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(54) Dna sequences encoding enzymes involved in production of isoprenoids

(57) The present invention is directed to an isolated DNA sequence coding for an enzyme involved in the mevalonate pathway or the pathway from isopentenyl pyrophosphate to farnesyl pyrophosphate, vectors or plasmids comprising such DNA, hosts transformed by either such DNAs or vectors or plasmids and a process for the production of isoprenoids and carotenoids by using such transformed host cells.

Description

[0001] The present invention relates to molecular biology for the manufacture of isoprenoids and biological materials useful therefor.

5 [0002] Astaxanthin is known to distribute in a wide variety of organisms such as animal (e.g. birds such as flamingo and scarlet ibis, and fish such as rainbow trout and salmon), algae and microorganisms. It is also recognized that astaxanthin has a strong antioxidation property against oxygen radical, which is expected to apply to pharmaceutical usage to protect living cells against some diseases such as a cancer. Moreover, from a viewpoint of industrial application, a demand for astaxanthin as a coloring reagent is increasing especially in the industry of farmed fish, such as salmon, 10 because astaxanthin imparts distinctive orange-red coloration to the animals and contributes to consumer appeal in the marketplace.

15 [0003] *Phaffia rhodozyma* is known as a carotenogenic yeast strain which produces astaxanthin specifically. Different from the other carotenogenic yeast, *Rhodotorula* species, *Phaffia rhodozyma* (*P. rhodozyma*) can ferment some sugars such as D-glucose. This is an important feature from a viewpoint of industrial application. In a recent taxonomic study, 20 a sexual cycle of *P. rhodozyma* was revealed and its telemorphic state was designated under the name of *Xanthophylomyces dendrorhous* (W.I. Golubev; Yeast 11, 101 - 110, 1995). Some strain improvement studies to obtain hyper producers of astaxanthin from *P. rhodozyma* have been conducted, but such efforts have been restricted to employ the method of conventional mutagenesis and protoplast fusion in this decade. Recently, Wery et al. developed a host vector system using *P. rhodozyma* in which a non-replicable plasmid was used to be integrated onto the genome of *P. rhodozyma* at the locus of ribosomal DNA in multcopies (Wery et al., Gene, 184, 89-97, 1997). And Verdoes et al. reported more improved vectors to obtain a transformant of *P. rhodozyma* as well as its three carotenogenic genes which code the enzymes that catalyzes the reactions from geranylgeranyl pyrophosphate to β-carotene (International patent WO97/23633). The importance of genetic engineering method on the strain improvement study of *P. rhodozyma* will increase in near future to break through the reached productivity by the conventional methods.

25 [0004] It is reported that the carotenogenic pathway from a general metabolite, acetyl-CoA consists of multiple enzymatic steps in carotenogenic eukaryotes as shown in Fig.1. Two molecules of acetyl-CoA are condensed to yield acetoacetyl-CoA which is converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by the action of 3-hydroxymethyl-3-glutaryl-CoA synthase. Next, 3-hydroxy-3-methylglutaryl-CoA reductase converts HMG-CoA to mevalonate, to which two molecules of phosphate residues are then added by the action of two kinases (mevalonate kinase and phosphomevalonate kinase). Mevalonate pyrophosphate is then decarboxylated by the action of mevalonate pyrophosphate decarboxylase to yield isopentenyl pyrophosphate (IPP) which becomes a building unit of wide varieties of isoprene molecules which is necessary in living organisms. This pathway is called as mevalonate pathway taken from its important intermediate, mevalonate. IPP is isomerized to dimethylallyl pyrophosphate (DMAPP) by the action of IPP isomerase. Then, IPP and DMAPP converted to C₁₀ unit, geranyl pyrophosphate (GPP) by the head to tail condensation. In a similar condensation reaction between GPP and IPP, GPP is converted to C₁₅ unit, farnesyl pyrophosphate (FPP) which is an important substrate of cholesterol in animal and ergosterol in yeast, and of farnesylation of regulation protein such as RAS protein. In general, the biosynthesis of GPP and FPP from IPP and DMAPP are catalyzed by one enzyme called FPP synthase (Laskovics et al., Biochemistry, 20, 1893-1901, 1981). On the other hand, in prokaryotes such as eubacteria, isopentenyl pyrophosphate was synthesized in a different pathway via 1-deoxyxylulose-5-phosphate from pyruvate which is absent in yeast and animal (Rohmer et al., Biochem. J., 295, 517-524, 1993). In exclusive studies of cholesterol biosynthesis, it was shown that rate-limiting steps of cholesterol metabolism were in the steps of this mevalonate pathway, especially in its early steps catalyzed by HMG-CoA synthase and HMG-CoA reductase. The inventors paid their attention to the fact that the biosynthetic pathways of cholesterol and carotenoid share their intermediate pathway from acetyl-CoA to FPP, and tried to improve the rate-limiting steps in the carotenogenic pathway 40 which might exist in the steps of mevalonate pathway, especially in early mevalonate pathway such as the steps catalyzed by HMG-CoA synthase and HMG-CoA reductase so as to improve the productivity of carotenoids, especially astaxanthin.

45 [0005] This invention is created based on the above endeavor of the inventors. In accordance with this invention, the genes and the enzymes involved in the mevalonate pathway from acetyl-CoA to FPP which are biological materials useful in the improvement of the astaxanthin production process are provided. This invention involves cloning and determination of the genes which code for HMG-CoA synthase, HMG-CoA reductase, mevalonate kinase, mevalonate pyrophosphate decarboxylase and FPP synthase. This invention also involves the enzymatic characterization as a result of the expression of such genes in suitable host organisms such as *E. coli*. These genes may be amplified in a suitable host, such as *P. rhodozyma* and their effects on the carotenogenesis can be confirmed by the cultivation of 50 such a transformant in an appropriate medium under an appropriate cultivation condition.

55 [0006] According to the present invention, there are provided an isolated DNA sequence coding for an enzyme involved in the mevalonate pathway or the reaction pathway from isopentenyl pyrophosphate to farnesyl pyrophosphate. More specifically, the said enzyme are those having an activity selected from the group consisting of 3-hydroxy-

3-methylglutaryl-CoA synthase activity, 3-hydroxy-3-methylglutaryl-CoA reductase activity, mevalonate kinase activity, mevalonate pyrophosphate decarboxylase activity and farnesyl pyrophosphate synthase.

[0007] The said isolated DNA sequence may be more specifically characterized in that (a) it codes for the said enzyme having an amino acid sequence selected from the group consisting of those described in SEQ ID NOs: 6, 7, 8,

5 9 and 10, or (b) it codes for a variant of the said enzyme selected from (i) an allelic variant, and (ii) an enzyme having one or more amino acid addition, insertion, deletion and/or substitution and having the stated enzyme activity. Particularly specified isolated DNA sequence mentioned above may be that which can be derived from a gene of *Phaffia rhodozyma* and is selected from (i) a DNA sequence represented in SEQ ID NOs: 1, 2, 4 or 5; (ii) an isocoding or an allelic variant for the DNA sequence represented in SEQ ID NOs: 1, 2, 4 or 5; and (iii) a derivative of a DNA sequence represented in SEQ ID NOs: 1, 2, 4 or 5 with addition, insertion, deletion and/or substitution of one or more nucleotide(s), and coding for a polypeptide having the said enzyme activity. Such derivatives can be made by recombinant means on the basis of the DNA sequences as disclosed herein by methods known in the state of the art and disclosed e.g. by Sambrook et al. (*Molecular Cloning, Cold Spring Harbour Laboratory Press, New York, USA, second edition 1989*). Amino acid exchanges in proteins and peptides which do not generally alter the activity are known in the state 10 of the art and are described, for example, by H. Neurath and R. L. Hill in *ÓThe ProteinsÓ* (Academic Press, New York, 1979, see especially Figure 6, page 14). The most commonly occurring exchanges are: Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, Asp/Gly, as well as these in reverse.

15 [0008] The present invention also provides an isolated DNA sequence, which is selected from (i) a DNA sequence represented in SEQ ID NO: 3; (ii) an isocoding or an allelic variant for the DNA sequence represented in SEQ ID NO: 3; and (iii) a derivative of a DNA sequence represented in SEQ ID NO: 3 with addition, insertion, deletion and/or substitution of one or more nucleotide(s), and coding for a polypeptide having the mevalonate kinase activity.

20 [0009] Furthermore the present invention is directed to those DNA sequences as specified above and as disclosed, e.g. in the sequence listing as well as their complementary strands, or those which include these sequences, DNA 25 sequences which hybridize under standard conditions with such sequences or fragments thereof and DNA sequences, which because of the degeneration of the genetic code, do not hybridize under standard conditions with such sequences but which code for polypeptides having exactly the same amino acid sequence.

20 [0010] "Standard conditions" for hybridization mean in this context the conditions which are generally used by a man skilled in the art to detect specific hybridization signals and which are described, e.g. by Sambrook et al., "Molecular Cloning" second edition, Cold Spring Harbour Laboratory Press 1989, New York, or preferably so called stringent hybridization and non-stringent washing conditions or more preferably so called stringent hybridization and stringent washing conditions a man skilled in the art is familiar with and which are described, e.g. in Sambrook et al. (s.a.). Furthermore DNA sequences which can be made by the polymerase chain reaction by using primers designed on the basis of the DNA sequences disclosed herein by methods known in the art are also an object of the present invention. It is understood that the DNA sequences of the present invention can also be made synthetically as described, e.g. in EP 35 747 483.

30 [0011] Further provided by the present invention is a recombinant DNA, preferably a vector and/or plasmid comprising a sequence coding for an enzyme functional in the mevalonate pathway or the reaction pathway from isopentenyl pyrophosphate to farnesyl pyrophosphate. The said recombinant DNA vector and/or plasmid may comprise the regulatory 40 regions such as promoters and terminators as well as open reading frames of above named DNAs.

40 [0012] The present invention also provides the use of the said recombinant DNA, vector or plasmid, to transform a host organism. The recombinant organism obtained by use of the recombinant DNA is capable of overexpressing DNA sequence encoding an enzyme involved in the mevalonate pathway or the reaction pathway from isopentenyl pyrophosphate to farnesyl pyrophosphate. The host organism transformed with the recombinant DNA may be useful in the 45 improvement of the production process of isoprenoids and carotenoids, in particular astaxanthin. Thus the present invention also provides such a recombinant organism/transformed host.

45 [0013] The present invention further provides a method for the production of isoprenoids or carotenoids, preferably carotenoids, which comprises cultivating thus obtained recombinant organism.

50 [0014] The present invention also relates to a method for producing an enzyme involved in the mevalonate pathway or the reaction pathway from isopentenyl pyrophosphate to farnesyl pyrophosphate, which comprises culturing a recombinant organism mentioned above, under a condition conductive to the production of said enzyme and relates also to the enzyme itself.

55 [0015] The present invention will be understood more easily on the basis of the enclosed figures and the more detailed explanations given below.

Fig. 1 depicts a scheme of deduced biosynthetic pathway from acetyl-CoA to astaxanthin in *P. rhodozyma*.

Fig. 2 shows the expression study by using an artificial *mvk* gene obtained from an artificial nucleotide addition at

amino terminal end of pseudo-*mvk* gene from *P. rhodozyma*. The cells from 50 µl of broth were subjected to 10 % sodium dodecyl sulfide - polyacrylamide gel electrophoresis (SDS-PAGE). Lane 1, *E. coli* (M15 (pREP4) (pQE30) without IPTG); Lane 2, *E. coli* (M15 (pREP4) (pQE30) with 1mM IPTG); Lane 3, Molecular weight marker (105 kDa, 82.0 kDa, 49.0 kDa, 33.3 kD and 28.6 kDa, up to down, BIO-RAD); Lane 4, *E. coli* (M15 (pREP4) (pMK1209 #3334) without IPTG); Lane 5, *E. coli* (M15 (pREP4) (pMK1209 #3334) with 1mM IPTG).

[0016] The present invention provides an isolated DNA sequence which code for enzymes which are involved in a biological pathway comprising the mevalonate pathway or the reaction pathway from isopentenyl pyrophosphate to farnesyl pyrophosphate. The said enzymes can be exemplified by those involved in the mevalonate pathway or the reaction pathway from isopentenyl pyrophosphate to farnesyl pyrophosphate in *Phaffia rhodozyma*, such as 3-hydroxy-3-methylglutaryl-CoA synthase, 3-hydroxy-3-methylglutaryl-CoA reductase, mevalonate kinase, mevalonate pyrophosphate decarboxylase and farnesyl pyrophosphate synthase. The present invention is useful for the production of the compounds involved in the biological pathway from the mevalonate pathway to the carotenogenic pathway and various products derived from such compounds. The compounds involved in the mevalonate pathway are acetoacetyl-CoA, 3-hydroxymethyl-3-glutaryl-CoA, mevalonic acid, mevalonate-phosphate, mevalonate-pyrophosphate and isopentenyl-pyrophosphate. Subsequently, isopentenyl-pyrophosphate is converted to geranylgeranyl-pyrophosphate through geranyl-pyrophosphate and farnesyl-pyrophosphate via the „Isoprene Biosynthesis” reactions as indicated in Fig. 1. The compounds involved in the carotenogenic pathway are geranylgeranyl-pyrophosphate, phytoene, lycopene, β-carotene and astaxanthin. Among the compounds involved in the above-mentioned biosynthesis, geranyl-pyrophosphate may be utilized for the production of ubiquinone. Farnesyl-pyrophosphate can be utilized for the production of sterols, such as cholesterol and ergosterol. Geranylgeranyl-pyrophosphate is a useful material for the production of vitamin K, vitamin E, chlorophyll and the like. Thus the present invention will be particularly useful when it is applied to a biological production of isoprenoids. Isoprenoids is the general term which collectively designates a series of compounds having isopentenyl-pyrophosphate as a skeleton unit. Further examples of isoprenoids are vitamin A and vitamin D₃.

[0017] The said DNA of the present invention can mean a cDNA which contains only open reading frame flanked between the short fragments in its 5'- and 3'- untranslated region and a genomic DNA which also contains its regulatory sequences such as its promoter and terminator which are necessary for the expression of the gene of interest.

[0018] In general, the gene consists of several parts which have different functions from each other. In eukaryotes, genes which encode corresponding protein are transcribed to premature messenger RNA (pre-mRNA) differing from the genes for ribosomal RNA (rRNA), small nuclear RNA (snRNA) and transfer RNA (tRNA). Although RNA polymerase II (PolII) plays a central role in this transcription event, PolII can not solely start transcription without *cis* element covering an upstream region containing a promoter and an upstream activation sequence (UAS), and a *trans*-acting protein factor. At first, a transcription initiation complex which consists of several basic protein components recognize the promoter sequence in the 5'-adjacent region of the gene to be expressed. In this event, some additional participants are required in the case of the gene which is expressed under some specific regulation, such as a heat shock response, or adaptation to a nutrition starvation, and so on. In such a case, a UAS is required to exist in the 5'-untranslated upstream region around the promoter sequence, and some positive or negative regulator proteins recognize and bind to the UAS. The strength of the binding of transcription initiation complex to the promoter sequence is affected by such a binding of the *trans*-acting factor around the promoter, and this enables the regulation of the transcription activity.

[0019] After the activation of a transcription initiation complex by the phosphorylation, a transcription initiation complex initiates transcription from the transcription start site. Some parts of the transcription initiation complex are detached as an elongation complex from the promoter region to the 3' direction of the gene (this step is called as a promoter clearance event) and an elongation complex continues the transcription until it reaches to a termination sequence that is located in the 3'-adjacent downstream region of the gene. Pre-mRNA thus generated is modified in nucleus by the addition of cap structure at the cap site which almost corresponds to the transcription start site, and by the addition of polyA stretches at the polyA signal which locates at the 3'-adjacent downstream region. Next, intron structures are removed from coding region and exon parts are combined to yield an open reading frame whose sequence corresponds to the primary amino acid sequence of a corresponding protein. This modification in which a mature mRNA is generated is necessary for a stable gene expression. cDNA in general terms corresponds to the DNA sequence which is reverse-transcribed from this mature mRNA sequence. It can be synthesized by the reverse transcriptase derived from viral species by using a mature mRNA as a template, experimentally.

[0020] To express a gene which was derived from eukaryote, a procedure in which cDNA is cloned into an expression vector in *E. coli* is often used as shown in this invention. This causes from a fact that a specificity of intron structure varies among the organisms and an inability to recognize the intron sequence from other species. In fact, prokaryote has no intron structure in its own genetic background. Even in the yeast, genetic background is different between ascomycetea to which *Saccharomyces cerevisiae* belongs and basidiomycetea to which *P. rhodozyma* belongs. Wery et al. showed that the intron structure of actin gene from *P. rhodozyma* cannot be recognized nor spliced by the ascomycetous yeast, *Saccharomyces cerevisiae* (Yeast, 12, 641-651, 1996).

[0021] Some other researchers reported that intron structures of some kinds of the genes involve regulation of their gene expressions (Dabeva, M. D. et al., Proc. Natl. Acad. Sci. U.S.A., 83, 5854, 1986). It might be important to use a genomic fragment which has its introns in a case of self-cloning of the gene of interest whose intron structure involves such a regulation of its own gene expression.

5 [0022] To apply a genetic engineering method for a strain improvement study, it is necessary to study its genetic mechanism in the event such as transcription and translation. It is important to determine a genetic sequence such as its UAS, promoter, intron structure and terminator to study the genetic mechanism.

[0023] According to this invention, the genes which code for the enzymes involving the mevalonate pathway were cloned from genomic DNA of *P. rhodozyma*, and their genomic sequence containing HMG-CoA synthase (*hmc*) gene, 10 HMG-CoA reductase (*hmg*) gene, mevalonate kinase (*mvk*) gene, mevalonate pyrophosphate decarboxylase (*mpd*) gene and FPP synthase (*fps*) gene including their 5'- and 3'-adjacent regions as well as their intron structures were determined.

[0024] At first, we cloned a partial gene fragment containing a portion of *hmc* gene, *hmg* gene, *mvk* gene, *mpd* gene and *fps* gene by using degenerate PCR method. The said degenerate PCR is a method to clone a gene of interest 15 which has high homology of amino acid sequence to the known enzyme from other species which has a same or similar function. Degenerate primer, which is used as a primer in degenerate PCR, was designed by a reverse translation of the amino acid sequence to corresponding nucleotides („degenerated”). In such a degenerate primer, a mixed primer which consists any of A, C, G or T, or a primer containing inosine at an ambiguity code is generally used. In this invention, such the mixed primers were used for degenerate primers to clone above genes. PCR condition used is varied 20 depending on primers and genes to clone as described hereinafter.

[0025] An entire gene containing its coding region with its intron as well as its regulation region such as a promoter or terminator can be cloned from a chromosome by screening of genomic library which is constructed in phage vector or plasmid vector in an appropriate host, by using a partial DNA fragment obtained by degenerate PCR as described above as a probe after it was labeled. Generally, *E. coli* as a host strain and *E. coli* vector, a phage vector such as λ 25 phage vector, or a plasmid vector such as pUC vector is often used in the construction of library and a following genetic manipulation such as a sequencing, a restriction digestion, a ligation and the like. In this invention, an *EcoRI* genomic library of *P. rhodozyma* was constructed in the derivatives of λ vector, λ ZAPII and λ DASHII depending on an insert size. An insert size, what length of insert must be cloned, was determined by the Southern blot hybridization for each gene 30 before a construction of a library. In this invention, a DNA which was used for a probe was labeled with digoxigenin (DIG), a steroid hapten instead of conventional ^{32}P label, following the protocol which was prepared by the supplier (Boehringer-Mannheim). A genomic library constructed from the chromosome of *P. rhodozyma* was screened by using a DIG-labeled DNA fragment which had a portion of a gene of interest as a probe. Hybridized plaques were picked up and used for further study. In the case of using λ DASHII (insert size was from 9 kb to 23 kb), prepared λ DNA was 35 digested by the *EcoRI*, followed by the cloning of the *EcoRI* insert into a plasmid vector such as pUC19 or pBluescriptII SK+. When λ ZAPII was used in the construction of the genomic library, *in vivo* excision protocol was conveniently used for the succeeding step of the cloning into the plasmid vector by using a derivative of single stranded M13 phage, Ex assist phage (Stratagene). A plasmid DNA thus obtained was examined for a sequencing.

[0026] In this invention, we used the automated fluorescent DNA sequencer, ALFred system (Pharmacia) using an autocycle sequencing protocol in which the Taq DNA polymerase is employed in most cases of sequencing.

40 [0027] After the determination of the genomic sequence, a sequence of a coding region was used for a cloning of cDNA of corresponding gene. The PCR method was also exploited to clone cDNA fragment. The PCR primers whose sequences were identical to the sequence at the 5'- and 3'- end of the open reading frame (ORF) were synthesized with an addition of an appropriate restriction site, and PCR was performed by using those PCR primers. In this invention, a cDNA pool was used as a template in this PCR cloning of cDNA. The said cDNA pool consists of various cDNA species 45 which were synthesized *in vitro* by the viral reverse transcriptase and Taq polymerase (CapFinder Kit manufactured by Clontech was used) by using the mRNA obtained from *P. rhodozyma* as a template. cDNA of interest thus obtained was confirmed in its sequence. Furthermore, cDNA thus obtained was used for a confirmation of its enzyme activity after the cloning of the cDNA fragment into an expression vector which functions in *E. coli* under the strong promoter activity such as the *lacZ* or *T7* expression system.

50 [0028] Succeeding to the confirmation of the enzyme activity, an expressed protein would be purified and used for raising of the antibody against the purified enzyme. Antibody thus prepared would be used for a characterization of the expression of the corresponding enzyme in a strain improvement study, an optimization study of the culture condition, and the like.

[0029] After the rate-limiting step is determined in the biosynthetic pathway which consists of multiple steps of enzymatic reactions, there are three strategies to enhance its enzymatic activity of the rate-limiting reaction by using its 55 genomic sequence.

[0030] One strategy is to use its gene itself as a native form. The simplest approach is to amplify the genomic sequence including its regulation sequence such as a promoter and a terminator. This is realized by the cloning of the

genomic fragment encoding the enzyme of interest into the appropriate vector on which a selectable marker that functions in *P. rhodozyma* is harbored. A drug resistance gene which encodes the enzyme that enables the host survive in the presence of a toxic antibiotic is often used for the selectable marker. G418 resistance gene harbored in pGB-Ph9 (Wery et al. (Gene, 184, 89-97, 1997)) is an example of a drug resistance gene. Nutrition complementation marker can be also used in the host which has an appropriate auxotrophy marker. *P. rhodozyma* ATCC24221 strain which requires cytidine for its growth is one example of the auxotroph. By using CTP synthetase as donor DNA for ATCC24221, a host vector system using a nutrition complementation can be established. As a vector, two types of vectors would be used. One of the vectors is an integrated vector which does not have an autonomous replicating sequence. Above pGB-Ph9 is an example of this type of a vector. Because such a vector does not have an autonomous replicating sequence in the vector, above vector cannot replicate by itself and can be present only in an integrated form on the chromosome of the host as a result of a single-crossing recombination using the homologous sequence between a vector and a chromosome. In case of increasing a dose of the integrated gene on the chromosome, amplification of the gene is often employed by using such a drug resistance marker. By increasing the concentration of the corresponding drug in the selection medium, the strain, in which the integrated gene is amplified on the chromosome as a result of recombination only can survive. By using such a selection, a strain which has amplified gene can be chosen. Another type of vector is a replicable vector which has an autonomous replicating sequence. Such a vector can exist in a multicopy state and this makes a dose of the harbored gene also exist in a multicopy state. By using such a strategy, an enzyme of interest which is coded by the amplified gene is expected to be overexpressed.

[0031] Another strategy to overexpress an enzyme of interest is a placement of a gene of interest under a strong promoter. In such a strategy, a copy number of a gene is not necessary to be in a multicopy state. This strategy is also applied to overexpress a gene of interest under the appropriate promoter whose promoter activity is induced in an appropriate growth phase and an appropriate timing of cultivation. Production of astaxanthin accelerates in a late phase of the growth such as the case of production of a secondary metabolite. Thus, the expression of carotenogenic genes may be maximized in a late phase of growth. In such a phase, gene expression of most biosynthesis enzyme decreases. For example, by placing a gene, which is involved in the biosynthesis of a precursor of astaxanthin and whose expression is under the control of a vegetative promoter such as a gene which encodes an enzyme which involves in mevalonate pathway, in the downstream of the promoter of carotenogenic genes, all the genes which are involved in the biosynthesis of astaxanthin become synchronized in their timings and phases of expression.

[0032] Still another strategy to overexpress enzymes of interest is induction of the mutation in its regulatory elements. For this purpose, a kind of reporter gene such as β -galactosidase gene, luciferase gene, a gene coding a green fluorescent protein, and the like is inserted between the promoter and the terminator sequence of the gene of interest so that all the parts including promoter, terminator and the reporter gene are fused and function each other. Transformed *P. rhodozyma* in which the said reporter gene is introduced on the chromosome or on the vector would be mutagenized *in vivo* to induce mutation within the promoter region of the gene of interest. Mutation can be monitored by detecting the change of the activity coded by the reporter gene. If the mutation occurs in a *cis* element of the gene, mutation point would be determined by the rescue of the mutagenized gene and sequencing. The determined mutation would be introduced to the promoter region on the chromosome by the recombination between a native promoter sequence and a mutated sequence. In the same procedure, the mutation occurring in the gene which encodes a *trans*-acting factor can be also obtained. It would also affect the overexpression of the gene of interest.

[0033] A mutation can be also induced by an *in vitro* mutagenesis of a *cis* element in the promoter region. In this approach, a gene cassette, containing a reporter gene which is fused to a promoter region derived from a gene of interest at its 5'-end and a terminator region from a gene of interest at its 3'-end, is mutagenized and then introduced into *P. rhodozyma*. By detecting the difference of the activity of the reporter gene, an effective mutation would be screened. Such a mutation can be introduced in the sequence of the native promoter region on the chromosome by the same method as the case of an *in vivo* mutation approach.

[0034] As a donor DNA, a gene which encodes an enzyme of mevalonate pathway or FPP synthase could be introduced solely or co-introduced by harboring on plasmid vector. A coding sequence which is identical to its native sequence, as well as its allelic variant, a sequence which has one or more amino acid additions, deletions and/or substitutions can be used as far as its corresponding enzyme has the stated enzyme activity. And such a vector can be introduced into *P. rhodozyma* by transformation and a transformant can be selected by spreading the transformed cells on an appropriate selection medium such as YPD agar medium containing geneticin in the case of pGB-Ph9 as a vector or a minimal agar medium omitting cytidine in the case of using auxotroph ATCC24221 as a recipient.

[0035] Such a genetically engineered *P. rhodozyma* would be cultivated in an appropriate medium and evaluated in its productivity of astaxanthin. A hyper producer of astaxanthin thus selected would be confirmed in view of the relationship between its productivity and the level of gene or protein expression which is introduced by such a genetic engineering method.

Examples

[0036] The following materials and methods were employed in the Example described below:

5 Strains

[0037] *P. rhodozyma* ATCC96594 (This strain has been redeposited on April 8, 1998 as a Budapest Treaty deposit under accession No. 74438).

10 *E. coli* DH5 α : F $^+$, ϕ 80d, lacZ Δ M15, Δ (lacZYA-argF)U169, hsd (r K^- , m K^+), recA1, endA1, deoR, thi-1, supE44, gyrA96, relA1 (Toyobo)

E. coli XL1-Blue MRF': Δ (mcrA)183, Δ (mcrCB-hsdSMR-mrr)173, endA1, supE44, thi-1, recA1, gyrA96, relA1, lac[F' proAB, lac $F^Z\Delta$ M15, Tn10 (tet r)] (Stratagene)

15 *E. coli* SOLR: e14 $^-$ (mcrA), Δ (mcrCB-hsdSMR-mrr)171, sbcC, recB, recJ, umuC :: Tn5(kan r), uvrC, lac, gyrA96, relA1, thi-1, endA1, λ^R , [F' proAB, lac $F^Z\Delta$ M15] Su $^+$ (nonsuppressing) (Stratagene, CA, USA)

20 *E. coli* XL1 MRA (P2): Δ (mcrA)183, Δ (mcrCB-hsdSMR-mrr)173, endA1, supE44, thi-1, gyrA96, relA1, lac (P2 lysogen) (Stratagene)

E. coli BL21 (DE3) (pLysS): dcm $^+$, ompTr B^- m B^- lon $^+$ λ (DE3), pLysS (Stratagene)

E. coli M15 (pREP4) (QIAGEN) (Zamenhof P. J. et al., J. Bacteriol. 110, 171-178, 1972)

25 *E. coli* KB822: pcnB80, zad :: Tn10, Δ (lacU169), hsdR17, endA1, thi-1, supE44

E. coli TOP10: F $^+$, mcrA, Δ (mrr-hsdRMS-mcrBC), ϕ 80, Δ lacZ M15, Δ lacX74, recA1, deoR, araD139, (ara-leu)7697, galU, galK, rpsL (Str r), endA1, nupG (Invitrogen)

30 Vectors

[0038]

35 λ ZAPII (Stratagene)

λ DASHII (Stratagene)

pBluescriptII SK+ (Stratagene)

40 pUC57 (MBI Fermentas)

pMOSBlue T-vector (Amersham)

45 pET4c (Stratagene)

pQE30 (QIAGEN)

pCR2.1TOPO (Invitrogen)

50 Media

[0039] *P. rhodozyma* strain is maintained routinely in YPD medium (DIFCO). *E. coli* strain is maintained in LB medium (10 g Bacto-trypton, 5 g yeast extract (DIFCO) and 5 g NaCl per liter). NZY medium (5 g NaCl, 2 g MgSO $_4 \cdot$ 7H $_2$ O, 5 g yeast extract (DIFCO), 10 g NZ amine type A (Sheffield) per liter) is used for λ phage propagation in a soft agar (0.7 % agar (WAKO)). When an agar medium was prepared, 1.5 % of agar (WAKO) was supplemented.

Methods

[0040] General methods of molecular genetics were practiced according to Molecular cloning: a Laboratory Manual, 2nd Edition (Cold Spring Harbor Laboratory Press, 1989). Restriction enzymes and T4 DNA ligase were purchased from Takara Shuzo (Japan).

[0041] Isolation of a chromosomal DNA from *P. rhodozyma* was performed by using QIAGEN Genomic Kit (QIAGEN) following the protocol supplied by the manufacturer. Mini-prep of plasmid DNA from transformed *E. coli* was performed with the Automatic DNA isolation system (PI-50, Kurabo, Co. Ltd., Japan). Midi-prep of plasmid DNA from an *E. coli* transformant was performed by using QIAGEN column (QIAGEN). Isolation of λ DNA was performed by Wizard lambda preps DNA purification system (Promega) following the protocol of the manufacturer. A DNA fragment was isolated and purified from agarose by using QIAquick or QIAEX II (QIAGEN). Manipulation of λ phage derivatives was done according to the protocol of the manufacturer (Stratagene).

[0042] Isolation of total RNA from *P. rhodozyma* was performed by the phenol method using Isogen (Nippon Gene, Japan). mRNA was purified from total RNA thus obtained by using mRNA separation kit (Clontech). cDNA was synthesized by using CapFinder cDNA construction kit (Clontech).

[0043] *In vitro* packaging was performed by using Gigapack III gold packaging extract (Stratagene).

[0044] Polymerase chain reaction (PCR) is performed with the thermal cycler from Perkin Elmer model 2400. Each PCR condition is described in examples. PCR primers were purchased from a commercial supplier or synthesized with a DNA synthesizer (model 392, Applied Biosystems). Fluorescent DNA primers for DNA sequencing were purchased from Pharmacia. DNA sequencing was performed with the automated fluorescent DNA sequencer (ALFred, Pharmacia).

[0045] Competent cells of DH5 α were purchased from Toyobo (Japan). Competent cells of M15 (pREP4) were prepared by CaCl₂ method as described by Sambrook et al. (Molecular cloning: a Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989).

25 Example 1 Isolation of mRNA from *P. rhodozyma* and construction of cDNA library

[0046] To construct cDNA library of *P. rhodozyma*, total RNA was isolated by phenol extraction method right after the cell disruption and the mRNA from *P. rhodozyma* ATCC96594 strain was purified by using mRNA separation kit (Clontech).

[0047] At first, Cells of ATCC96594 strain from 10 ml of two-day-culture in YPD medium were harvested by centrifugation (1500 x g for 10 min.) and washed once with extraction buffer (10 mM Na-citrate / HCl (pH 6.2) containing 0.7 M KCl). After suspending in 2.5 ml of extraction buffer, the cells were disrupted by French press homogenizer (Ohtake Works Corp., Japan) at 1500 kgf/cm² and immediately mixed with two times of volume of isogen (Nippon gene) according to the method specified by the manufacturer. In this step, 400 μ g of total RNA was recovered.

[0048] Then this total RNA was purified by using mRNA separation kit (Clontech) according to the method specified by the manufacturer. Finally, 16 μ g of mRNA from *P. rhodozyma* ATCC96594 strain was obtained.

[0049] To construct cDNA library, CapFinder PCR cDNA construction kit (Clontech) was used according to the method specified by the manufacturer. One μ g of purified mRNA was applied for a first strand synthesis followed by PCR amplification. After this amplification by PCR, 1 mg of cDNA pool was obtained.

Example 2 Cloning of the partial *hmc* (3-hydroxy-3-methylglutaryl-CoA synthase) gene from *P. rhodozyma*

[0050] To clone a partial *hmc* gene from *P. rhodozyma*, a degenerate PCR method was exploited. Two mixed primers whose nucleotide sequences were designed and synthesized as shown in TABLE 1 based on the common sequence of known HMG-CoA synthase genes from other species.

TABLE 1

50 Sequence of primers used in the cloning of *hmc* gene

Hmgs1 : GGNAARTAYACNATHGGNYTNGGNCA (sense primer) (SEQ ID NO: 11)
Hmgs3 : TANARNSWNSWNGTRTACATRTTNCC (antisense primer) (SEQ ID NO: 12)
(N=A, C, G or T; R=A or G, Y=C or T, H=A, T or C, S=C or G, W=A or T)

55

[0051] After the PCR reaction of 25 cycles of 95 °C for 30 seconds, 50 °C for 30 seconds and 72°C for 15 seconds by using ExTaq (Takara Shuzo) as a DNA polymerase and cDNA pool obtained in example 1 as a template, reaction

mixture was applied to agarose gel electrophoresis. A PCR band that has a desired length was recovered and purified by QIAquick (QIAGEN) according to the method by the manufacturer and then ligated to pMOSBlue T-vector (Amersham). After the transformation of competent *E. coli* DH5 α , 6 white colonies were selected and plasmids were isolated with Automatic DNA isolation system. As a result of sequencing, it was found that 1 clone had a sequence whose deduced amino acid sequence was similar to known *hmc* genes. This isolated cDNA clone was designated as pHMC211 and used for further study.

Example 3 Isolation of genomic DNA from *P. rhodozyma*

[0052] To isolate a genomic DNA from *P. rhodozyma*, QIAGEN genomic kit was used according to the method specified by the manufacturer.
[0053] At first, cells of *P. rhodozyma* ATCC96594 strain from 100 ml of overnight culture in YPD medium were harvested by centrifugation (1500 x g for 10 min.) and washed once with TE buffer (10 mM Tris / HCl (pH 8.0) containing 1 mM EDTA). After suspending in 8 ml of Y1 buffer of the QIAGEN genomic kit, lyticase (SIGMA) was added at the concentration of 2 mg/ml to disrupt cells by enzymatic degradation and the reaction mixture was incubated for 90 minutes at 30 °C and then proceeded to the next extraction step. Finally, 20 µg of genomic DNA was obtained.

Example 4 Southern blot hybridization by using pHMC211 as a probe

[0054] Southern blot hybridization was performed to clone a genomic fragment which contains *hmc* gene from *P. rhodozyma*. Two µg of genomic DNA was digested by *Eco*RI and subjected to agarose gel electrophoresis followed by acidic and alkaline treatment. The denatured DNA was transferred to nylon membrane (Hybond N+, Amersham) by using transblot (Joto Rika) for an hour. The DNA which was transferred to nylon membrane was fixed by a heat treatment (80 °C, 90 minutes). A probe was prepared by labeling a template DNA (*Eco*RI- and *Sall*- digested pHMC211) with DIG multiprimer method (Boehringer Mannheim). Hybridization was performed with the method specified by the manufacturer. As a result, hybridized band was visualized in the range from 3.5 to 4.0 kilobases (kb).

Example 5 Cloning of a genomic fragment containing *hmc* gene

[0055] Four µg of the genomic DNA was digested by *Eco*RI and subjected to agarose gel electrophoresis. Then, DNAs whose length is within the range from 3.0 to 5.0 kb was recovered by QIAEX II gel extraction kit (QIAGEN) according to the method specified by the manufacturer. The purified DNA was ligated to 1 µg of *Eco*RI-digested and CIAP (calf intestine alkaline phosphatase) -treated λZAPII (Stratagene) at 16 °C overnight, and packaged by Gigapack III gold packaging extract (Stratagene). The packaged extract was infected to *E. coli* XL1Blue MRF' strain and over-laid with NZY medium poured onto LB agar medium. About 6000 plaques were screened by using *Eco*RI- and *Sall*- digested pHMC211 as a probe. Two plaques were hybridized to the labeled probe and subjected to *in vivo* excision protocol according to the method specified by the manufacturer (Stratagene). It was found that isolated plasmids had the same fragments in the opposite direction each other as results of restriction analysis and sequencing. As a result of sequencing, the obtained *Eco*RI fragment contained same nucleotide sequence as that of pHMC211 clone. One of these plasmids was designated as pHMC526 and used for further study. A complete nucleotide sequence was obtained by sequencing of deletion derivatives of pHMC526, and sequencing with a primer-walking procedure. The insert fragment of pHMC526 consists of 3431 nucleotides that contained 10 complete and an incomplete exons and 10 introns with about 1 kb of 3'-terminal untranslated region.

Example 6 Cloning of upstream region of *hmc* gene

[0056] Cloning of 5'- adjacent region of *hmc* gene was performed by using Genome Walker Kit (Clontech), because pHMC 526 does not contain its 5' end of *hmc* gene. At first, the PCR primers whose sequences were shown in TABLE 2 were synthesized.

TABLE 2

Sequence of primers used in the cloning of 5'- adjacent region of *hmc* gene

Hmc21 ; GAAGAACCCCATAAAAGCCTCGA (primary primer) (SEQ ID NO: 13)

Hmc22 ; AAAAGCCTCGAGATCCTTGTGAGCG (nested primer) (SEQ ID NO: 14)

EP 0 955 363 A2

[0057] Protocols for library construction and PCR condition were the same as those specified by the manufacturer by using the genomic DNA preparation obtained in Example 3 as a PCR template. The PCR fragments that had *EcoRV* site at the 5' end (0.45 kb), and that had *PvuII* site at the 5' end (2.7 kb) were recovered and cloned into pMOSBlue T-vector by using *E. coli* DH5 α as a host strain. As a result of sequencing of each 5 of independent clones from both constructs, it was confirmed that the 5' adjacent region of *hmc* gene was cloned and small part (0.1 kb) of *EcoRI* fragment within its 3' end was found. The clone obtained by the *PvuII* construct in the above experiment was designated as pHMCPv708 and used for further study.

[0058] Next, Southern blot analysis was performed by the method as shown in above Example 4, and 5'-adjacent region of the *hmc* gene existed in 3 kb of *EcoRI* fragment was determined. After a construction from 2.5 to 3.5 kb *EcoRI* library in λ ZAPII, 600 plaques were screened and 6 positive clones were selected. As a result of sequencing of these 6 clones, it was clarified that 4 clones within 6 positive plaques had the same sequence as that of the pHMCPv708, and one of those was named as pHMC723 and used for further analysis.

[0059] The PCR primers whose sequences were shown in TABLE 3 were synthesized to clone small (0.1 kb) *EcoRI* fragment locating between 3.5 kb and 3.0 kb *EcoRI* fragments on the chromosome of *P. rhodozyma*.

15

TABLE 3

Sequence of primers used in the cloning small *EcoRI* portion of *hmc* gene.

20 Hmc30 : AGAAGCCAGAAGAGAAAA (sense primer) (SEQ ID NO: 15)

Hmc31 : TCGTCGAGGAAAGTAGAT (antisense primer) (SEQ ID NO: 16)

[0060] The PCR condition was the same as shown in Example 2. Amplified fragment (0.1 kb in its length) was cloned 25 into pMOSBlue T-vector and transformed *E. coli* DH5 α . Plasmids were prepared from 5 independent white colonies and subjected to the sequencing.

[0061] Thus, it was determined that the nucleotide sequence (4.8 kb) contained *hmc* gene (SEQ ID NO: 1). Coding region was in 2432 base pairs that consisted of 11 exons and 10 introns. Introns were scattered all through the coding region without 5' or 3' bias. It was found that open reading frame consists of 467 amino acids (SEQ ID NO: 6) whose 30 sequence is strikingly similar to the known amino acid sequence of HMG-CoA synthase gene from other species (49.6 % identity to HMG-CoA synthase from *Schizosaccharomyces pombe*).

Example 7 Expression of *hmc* gene in *E. coli* and confirmation of its enzymatic activity

35 [0062] The PCR primers whose sequences were shown in TABLE 4 were synthesized to clone a cDNA fragment of *hmc* gene.

40

TABLE 4

Sequence of primers used in the cloning of cDNA of *hmc* gene

Hmc25 : GGTACCATATGTATCCTTCTACTACCGAAC (sense primer) (SEQ ID NO: 17)

Hmc26 : GCATGCGGATCCTCAAGCAGAAGGGACCTG (antisense primer) (SEQ ID NO: 18)

45

[0063] PCR condition was as follows; 25 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 3 minutes. As a template, 0.1 μ g of cDNA pool obtained in Example 2 was used, and Pfu polymerase as a DNA polymerase. Amplified 1.5 kb fragment was recovered and cloned in pT7Blue-3 vector (Novagen) by using perfectly blunt cloning kit (Novagen) according to the protocol specified by the manufacturer. Six independent clones from white colonies of *E. coli* 50 DH5 α transformants were selected and plasmids were prepared from those transformants. As a result of restriction analysis, 2 clones were selected for a further selection by sequencing. One clone has an amino acid substitution at position 280 (from glycine to alanine) and another has at position 53 (from alanine to threonine). Alignment of an amino acid sequences derived from known *hmc* genes showed that alanine residue as well as glycine residue at position 280 was observed well in all the sequences from other species and this fact suggested that amino acid substitution at position 55 280 would not affect its enzymatic activity. This clone (mutant at position 280) was selected as pHMC731 for a succeeding expression experiment.

[0064] Next, 1.5 kb fragment obtained by *NdeI*- and *BamHI*-digestion of pHMC731 was ligated to pET11c (StrataGene) digested by the same pairs of restriction enzymes, and introduced to *E. coli* DH5 α . As a result of restriction anal-

ysis, plasmid that had a correct structure (pHMC818) was recovered. Then, competent *E. coli* BL21 (DE3) (pLysS) cells (Stratagene) were transformed, and one clone that had a correct structure was selected for further study.

[0065] For an expression study, strain BL21 (DE3) (pLysS) (pHMC818) and vector control strain BL21 (DE3) (pLysS) (pET11c) were cultivated in 100 ml of LB medium at 37 °C until OD at 600 nm reached to 0.8 (about 3 hours) in the presence of 100 µg/ml of ampicillin. Then, the broth was divided in two portions of the same volume, and then 1 mM of isopropyl β-D-thiogalactopyranoside (IPTG) was added to one portion. Cultivation was continued for further 4 hours at 37 °C. Twenty five µl of broth was removed from induced- and uninduced- culture of *hmc* clone and vector control cultures and subjected to sodium dodesyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) analysis. It was confirmed that protein whose size was similar to deduced molecular weight from nucleotide sequence (50.8 kDa) was expressed only in the case of clone that harbored pHMC818 with the induction. Cells from 50 ml broth were harvested by the centrifugation (1500 x g, 10 minutes), washed once and suspended in 2 ml of hmc buffer (200 mM Tris-HCl (pH 8.2)). Cells were disrupted by French press homogenizer (Ohtake Works) at 1500 kgf/cm² to yield a crude lysate. After the centrifugation of the crude lysate, a supernatant fraction was recovered and used as a crude extract for an enzymatic analysis. In the only case of induced lysate of pHMC818 clone, a white pellet was spun down and was recovered.

15 Enzyme assay for 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase was performed by the photometric assay according to the method by Stewart *et al.* (J. Biol. Chem. 241(5), 1212-1221, 1966). In all the crude extract, the activity of 3-hydroxy-3-methylglutaryl-CoA synthase was not detected. As a result of SDS-PAGE analysis of the crude extract, an expressed protein band that had found in expressed broth was disappeared. Subsequently the white pellet that was recovered from the crude lysate of induced pHMC818 clone was solubilized with 8 M guanidine-HCl, and then subjected to SDS-PAGE analysis. The expressed protein was recovered in the white pellet, and this suggested that expressed protein would form an inclusion body.

[0066] Next, an expression experiment in more mild condition was conducted. Cells were grown in LB medium at 28 °C and the induction was performed by the addition of 0.1 mM of IPTG. Subsequently, incubation was continued further for 3.5 hours at 28 °C and then the cells were harvested. Preparation of the crude extract was the same as the previous protocol. Results are summarized in TABLE 5. It was shown that HMG-CoA synthase activity was only observed in the induced culture of the recombinant strain harboring *hmc* gene, and this suggested that the cloned *hmc* gene encodes HMG-CoA synthase.

TABLE 5

Enzymatic characterization of <i>hmc</i> cDNA clone		
plasmid	IPTG	µ mol of HMG-CoA / minute / mg-protein
pHMC818	-	0
	+	0.146
pET11c	-	0
	+	0

Example 8 Cloning of *hmg* (3-hydroxymethyl-3-glutaryl-CoA reductase) gene

[0067] Cloning protocol of *hmg* gene was almost the same as the *hmc* gene shown in Example 2 to 7. At first, the PCR primers whose sequences were shown in TABLE 6 based on the common sequences of HMG-CoA reductase genes from other species were synthesized.

TABLE 6

50 Sequence of primers used in the cloning of *hmg* gene

Red1 ; GCNTGYTGYGARAAYGTNATHGGNTAYATGCC (sense primer) (SEQ ID NO: 19)

Red2 ; ATCCARTTDATNGCNGCNGGYTTTTCNGT (antisense primer) (SEQ ID NO: 20)

55 (N=A, C, G or T; R=A or G, Y=C or T, H=A, T or C, D=A, G or T)

[0068] After the PCR reaction of 25 cycles of 95 °C for 30 seconds, 54 °C for 30 seconds and 72°C for 30 seconds by using ExTaq (Takara Shuzo) as a DNA polymerase, reaction mixture was applied to agarose gel electrophoresis.

PCR band that has a desired length was recovered and purified by QIAquick (QIAGEN) according to the method by the manufacturer and then ligated to pUC57 vector (MBI Fermentas). After the transformation of competent *E. coli* DH5 α , 7 white colonies were selected and the plasmids were isolated from those transformants. As a result of sequencing, it was found that all the clones had a sequence whose deduced amino acid sequence was similar to known HMG-CoA reductase genes. One of the isolated cDNA clones was named as pRED1219 and used for further study.

[0069] Next, a genomic fragment containing 5'- and 3'- adjacent region of *hmg* gene was cloned with the Genome Walker kit (Clontech). The 2.5 kb fragment of 5' adjacent region (pREDPVu1226) and 4.0 kb fragment of 3' adjacent region of *hmg* gene (pREDEVd1226) were cloned. Based on the sequence of the insert of pREDPVu1226, PCR primers whose sequence were shown in TABLE 7 were synthesized.

10

TABLE 7

Sequence of primers used in the cloning of cDNA of *hmg* gene

15	Red8 : GGCCATTCCACACTTGATGCTCTGC (antisense primer) (SEQ ID NO: 21)
	Red9 : GGCGATATCTTATGGTCCT (sense primer) (SEQ ID NO: 22)

[0070] Subsequently a cDNA fragment containing a long portion of *hmg* cDNA sequence was cloned by a PCR method by using Red 8 and Red 9 as PCR primers and the cDNA pool prepared in Example 2 and thus cloned plasmid was named as pRED107. PCR condition was as follows; 25 cycles of 30 seconds at 94 °C, 30 seconds at 55 °C and 1 minute at 72 °C.

[0071] Southern blot hybridization study was performed to clone genomic sequence which contains the entire *hmg* gene from *P. rhodozyma*. Probe was prepared by labeling a template DNA, pRED107 with DIG multiprimer method. Hybridization was performed with the method specified by the manufacturer. As a result, labeled probe hybridized to two bands that had 12 kb and 4 kb in their lengths. As a result of sequencing of pREDPVu1226, EcoRI site wasn't found in the cloned *hmg* region. This suggested that another species of *hmg* gene (that has 4 kb of hybridized EcoRI fragment) existed on the genome of *P. rhodozyma* as found in other organisms.

[0072] Next, a genomic library consisting of 9 to 23 kb of EcoRI fragment in the λ DASHII vector was constructed. The packaged extract was infected to *E. coli* XL1 Blue, MRA(P2) strain (Stratagene) and over-laid with NZY medium poured onto LB agar medium. About 5000 plaques were screened by using 0.6 kb fragment of *Stu*I- digested pRED107 as a probe. 4 plaques were hybridized to the labeled probe. Then a phage lysate was prepared and DNA was purified with Wizard lambda purification system according to the method specified by the manufacturer (Promega) and was digested with EcoRI to isolate 10 kb of EcoRI fragment and to clone in EcoRI-digested and CIAP-treated pBluescriptII KS-(Stratagene). Eleven white colonies were selected and subjected to a colony PCR by using Red9 and -40 universal primer (Pharmacia). Template DNA for a colony PCR was prepared by heating cell suspension in which picked-up colony was suspended in 10 μ l of sterilized water for 5 minutes at 99 °C prior to a PCR reaction (PCR condition; 25 cycles of 30 seconds at 94 °C, 30 seconds at 55 °C and 3 minutes at 72 °C). One colony gave 4 kb of a positive PCR band, and it suggested that this clone had an entire region containing *hmg* gene. A plasmid from this positive clone was prepared and named as pRED611. Subsequently deletion derivatives of pRED611 were made up for sequencing. By combining the sequence obtained from deletion mutants with the sequence obtained by a primer-walking procedure, the nucleotide sequence of 7285 base pairs which contains *hmg* gene from *P. rhodozyma* was determined (SEQ ID NO: 2). The *hmg* gene from *P. rhodozyma* consists of 10 exons and 9 introns. The deduced amino acid sequence of 1092 amino acids in its length (SEQ ID NO: 7) showed an extensive homology to known HMG-CoA reductase (53.0 % identity to HMG-CoA reductase from *Ustilago maydis*).

Example 9 Expression of carboxyl-terminal domain of *hmg* gene in *E. coli*

[0073] Some species of prokaryotes have soluble HMG-CoA reductases or related proteins (Lam et al., J. Biol. Chem. 267, 5829-5834, 1992). However, in eukaryotes, HMG-CoA reductase is tethered to the endoplasmic reticulum via an amino-terminal membrane domain (Skalnik et al., J. Biol. Chem. 263, 6836-6841, 1988). In fungi (i.e. *Saccharomyces cerevisiae* and the smut fungus, *Ustilago maydis*) and in animals, the membrane domain is large and complex, containing seven or eight transmembrane segments (Croxen et al. Microbiol. 140, 2363-2370, 1994). In contrast, the membrane domains of plant HMG-CoA reductase proteins have only one or two transmembrane segments (Nelson et al. Plant Mol. Biol. 25, 401-412, 1994). Despite the difference in the structure and sequence of the transmembrane domain, the amino acid sequences of the catalytic domain are conserved across eukaryotes, archaebacteria and eubacteria.

[0074] Croxen et al. showed that C-terminal domain of HMG-CoA reductase derived from the maize fungal pathogen,

Ustilago maydis was expressed in active form in *E. coli* (Microbiology, 140, 2363-2370, 1994). The inventors of the present invention tried to express a C-terminal domain of HMG-CoA reductase from *P. rhodozyma* in *E. coli* to confirm its enzymatic activity.

[0075] At first, the PCR primers whose sequences were shown in TABLE 8 were synthesized to clone a partial cDNA fragment of *hmg* gene. The sense primer sequence corresponds to the sequence which starts from 597th amino acid (glutamate) residue, and a length of protein and cDNA which was expected to obtain was 496 amino acids and 1.5 kb, respectively.

10 TABLE 8

Sequence of primers used in the cloning of a partial cDNA of *hmg* gene

Red54 ; GGTACCGAAGAAATTATGAAGAGTGG (sense primer) (SEQ ID NO: 23)

15 Red55 ; CTGCAGTCAGGCATCCACGTTCACAC (antisense primer) (SEQ ID NO: 24)

[0076] The PCR condition was as follows; 25 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 3 minutes. As a template, 0.1 µg of cDNA pool obtained in Example 2 and as a DNA polymerase, ExTaq polymerase were used. Amplified 1.5 kb fragment was recovered and cloned in pMOSBlue T-vector (Novagen). Twelve independent clones from white colonies of *E. coli* DH5 α transformants were selected and plasmids were prepared from those transformants. As a result of restriction analysis, all the clones were selected for a further selection by sequencing. One clone did not have an amino acid substitution all through the coding sequence and was named as pRED908.

[0077] Next, 1.5 kb fragment obtained by *Kpn*I- and *Pst*I- digestion of pRED908 was ligated to pQE30 (QIAGEN) digested by the same pairs of restriction enzymes, and transformed to *E. coli* KB822. As a result of restriction analysis, 25 plasmid that had a correct structure (pRED1002) was recovered. Then, competent *E. coli* M15 (pREP4) cells (QIAGEN) were transformed and one clone that had a correct structure was selected for further study.

[0078] For an expression study, strain M15 (pREP4) (pRED1002) and vector control strain M15 (pREP4) (pQE30) were cultivated in 100 ml of LB medium at 30 °C until OD at 600 nm reached to 0.8 (about 5 hours) in the presence of 25 µg/ml of kanamycin and 100 µg/ml of ampicillin. Then, the broth was divided into two portions of the same volume, 30 and then 1 mM of IPTG was added to one portion. Cultivation continued for further 3.5 hours at 30 °C. Twenty five µl of the broth was removed from induced- and uninduced- culture of *hmg* clone and vector control cultures and subjected to SDS-PAGE analysis. It was confirmed that protein whose size was similar to deduced molecular weight from nucleotide sequence (52.4 kDa) was expressed only in the case of clone that harbored pRED1002 with the induction. Cells from 50 ml broth were harvested by the centrifugation (1500 x g, 10 minutes), washed once and suspended in 2 ml of 35 *hmg* buffer (100 mM potassium phosphate buffer (pH 7.0) containing 1 mM of EDTA and 10 mM of dithiothreitol). Cells were disrupted by French press (Ohtake Works) at 1500 kgf/cm² to yield a crude lysate. After the centrifugation of the crude lysate, a supernatant fraction was recovered and used as a crude extract for enzymatic analysis. In the only case of induced lysate of pRED1002 clone, a white pellet was spun down and was recovered. Enzyme assay for 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase was performed by the photometric assay according to the method by 40 Servouse et al. (Biochem. J. 240, 541-547, 1986). In all the crude extract, the activity of 3-hydroxy-3-methylglutaryl-CoA synthase was not detected. As a result of SDS-PAGE analysis for the crude extract, expressed protein band that had found in expressed broth was disappeared. Next, the white pellet that was recovered from the crude lysate of induced pRED1002 clone was solubilized with an equal volume of 20 % SDS, and then subjected to SDS-PAGE analysis. An expressed protein was recovered in the white pellet, and this suggested that the expressed protein would form 45 an inclusion body.

[0079] Next, the expression experiment was performed in more mild condition. Cells were grown in LB medium at 28 °C and the induction was performed by the addition of 0.1 mM of IPTG. Then, incubation was continued further for 3.5 hours at 28 °C and then the cells were harvested. Preparation of the crude extract was the same as the previous protocol. Results are summarized in TABLE 9. It was shown that 30 times higher induction was observed, and this suggested that the cloned *hmg* gene codes HMG-CoA reductase.

TABLE 9

Enzymatic characterization of <i>hmg</i> cDNA clone		
plasmid	IPTG μ mol of NADPH / minute	/ mg-protein
pRED1002	-	0.002
	+	0.059
pQE30	-	0
	+	0

Example 10 Cloning of mevalonate kinase (*mvk*) gene

[0080] A cloning protocol of *mvk* gene was almost the same as the *hmc* gene shown in Example 2 to 7. At first, the PCR primers whose sequence were shown in TABLE 10, based on the common sequences of mevalonate kinase genes from other species were synthesized.

TABLE 10

Sequence of primers used in the cloning of *mvk* gene

Mk1 ; GCNCCNGGNAARGTNATHYNTTYGGNGA (sense primer) (SEQ ID NO: 25)
Mk2 ; CCCCANGTNSWNACNGCRTTRTCNACNCC (antisense primer) (SEQ ID NO: 26)
(N=A, C, G or T; R=A or G, Y=C or T, H=A, T or C, S=C or G, W=A or T)

[0081] After the PCR reaction of 25 cycles of 95 °C for 30 seconds, 46 °C for 30 seconds and 72°C for 15 seconds by using ExTaq as a DNA polymerase, the reaction mixture was applied to agarose gel electrophoresis. A 0.6 kb of PCR band whose length was expected to contain a partial *mvk* gene was recovered and purified by QIAquick according to the method indicated by the manufacturer and then ligated to pMOSBlue T-vector. After a transformation of competent *E. coli* DH5 α cells, 4 white colonies were selected and plasmids were isolated. As a result of sequencing, it was found that one of the clones had a sequence whose deduced amino acid sequence was similar to known mevalonate kinase genes. This cDNA clone was named as pMK128 and used for further study.

[0082] Next, a partial genomic clone which contained *mvk* gene was cloned by PCR. The PCR primers whose sequence were shown in TABLE 11, based on the internal sequence of pMK128 were synthesized.

TABLE 11

Sequence of primers used in the cloning of genomic DNA containing *mvk* gene

Mk5 ; ACATGCTGTAGTCCATG (sense primer) (SEQ ID NO: 27)
Mk6 ; ACTCGGATTCCATGGA (antisense primer) (SEQ ID NO: 28)

[0083] PCR condition was 25 cycles of 30 seconds at 94 °C, 30 seconds at 55 °C and 1 minute at 72 °C. The amplified 1.4 kb fragment was cloned into pMOSBlue T-vector. As a result of sequencing, it was confirmed a genomic fragment containing *mvk* gene which had typical intron structures could be obtained and this genomic clone was named as pMK224.

[0084] Southern blot hybridization study was performed to clone a genomic fragment which contained an entire *mvk* gene from *P. rhodozyma*. Probe was prepared by labeling a template DNA, pMK224 digested by *Nco*I with DIG multipriming method. Hybridization was performed with the method specified by the manufacturer. As a result, the labeled probe hybridized to a band that had 6.5 kb in its lengths. Next, a genomic library consisting of 5 to 7 kb of *Eco*RI fragment was constructed in the λ ZAPII vector. The packaged extract was infected to *E. coli* XL1Blue, MRF strain (Strata-

gene) and over-laid with NZY medium poured onto LB agar medium. About 5000 plaques were screened by using 0.8 kb fragment of *Nco*I-digested pMK224 as a probe. Seven plaques were hybridized to the labeled probe. Then a phage lysate was prepared according to the method specified by the manufacturer (Stratagene) and *in vivo* excision was performed by using *E. coli* XL1Blue MRF' and SOLR strains. Fourteen white colonies were selected and plasmids were isolated from those selected transformants. Then, isolated plasmids were digested by *Nco*I and subjected to Southern blot hybridization with the same probe as the plaque hybridization. The insert fragments of all the plasmids were hybridized to the probe and this suggested that a genomic fragment containing *mvk* gene could be cloned. A plasmid from one of the positive clones was prepared and was named as pMK701. About 3 kb of sequence was determined by the primer walking procedure and it was revealed that 5' end of the *mvk* gene wasn't included into pMK701.

5 [0085] Next, a PCR primer which had a sequence :

TTGTTGTCGTAGCAGTGGGTGAGAG (SEQ ID NO: 29) was synthesized to clone the 5'-adjacent genomic region of *mvk* gene with the Genome Walker Kit according to the method specified by manufacturer (Clontech). A specific 1.4 kb PCR band was amplified and cloned into pMOSBlue T-vector. All of the transformants of DH5 α selected had expected length of the insert. Subsequent sequencing revealed that 5'-adjacent region of *mvk* gene could be cloned. One of the 15 clone was designated as pMKEVR715 and used for further study. As a result of Southern blot hybridization using genomic DNA prepared in example 3, the labeled pMKEVR715 hybridized to 2.7 kb *Eco*RI band. Then a genomic library in which *Eco*RI fragments from 1.4 to 3.0 kb in lengths were cloned into λ ZAPII was constructed and screened with 1.0 kb of *Eco*RI fragment from pMKEVR715. Fourteen positive plaques were selected from 5000 plaques and plasmids were prepared from those plaques with *in vivo* excision procedure.

20 [0086] The PCR primers whose sequences were shown in TABLE 12, taken from the internal sequence of pMKEVR715 were synthesized to select a positive clone with a colony PCR.

TABLE 12

25 PCR primers used for colony PCR to clone 5'-adjacent region of *mvk* gene

Mk17 ; GGAAGAGGAAGAGAAAAG (sense primer) (SEQ ID NO: 30)

Mk18 ; TTGCCGAACTCAATGTAG (antisense primer) (SEQ ID NO: 31)

30 [0087] PCR condition was as follows; 25 cycles of 30 seconds at 94 °C, 30 seconds at 50 °C and 15 seconds at 72 °C. From all the candidates except one clone, the positive 0.5 kb band was yielded. One of the clones was selected and named as pMK723 to determine the sequence of the upstream region of *mvk* gene. After sequencing of the 3'-region of pMK723 and combining with the sequence of pMK701, the genomic sequence of 4.8 kb fragment containing *mvk* gene was determined. The *mvk* gene consists of 4 introns and 5 exons (SEQ ID NO: 3). The deduced amino acid sequence except 4 amino acids in the amino terminal end (SEQ ID NO: 8) showed an extensive homology to known mevalonate kinase (44.3 % identity to mevalonate kinase from *Rattus norvegicus*).

Example 11 Expression of *mvk* gene by the introduction of 1 base at amino terminal region

40 [0088] Although the amino acid sequence showed a significant homology to known mevalonate kinase, an appropriate start codon for *mvk* gene could not be found. This result suggested the cloned gene might be a pseudogene for mevalonate kinase. To confirm this assumption, PCR primers whose sequences are shown in TABLE 13 were synthesized to introduce an artificial nucleotide which resulted in the generation of appropriate start codon at the amino terminal end.

TABLE 13

50 PCR primers used for the introduction of a nucleotide into *mvk* gene

Mk33 ; GGATCCATGAGAGCCCCAAAAAGAAGA (sense primer) (SEQ ID NO: 32)

Mk34 ; GTCGACTCAAGCAAAAGACCAACGAC (antisense primer) (SEQ ID NO: 33)

55 [0089] The artificial amino terminal sequence thus introduced were as follows; NH2-Met-Arg-Ala-Gln. After the PCR reaction of 25 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30 seconds by using ExTaq polymerase as a DNA polymerase. The reaction mixture was subjected to agarose gel electrophoresis. An expected 1.4 kb of PCR band was amplified and cloned into pCR2.1 TOPO vector. After a transformation of competent *E. coli* TOP10 cells,

6 white colonies were selected and plasmids were isolated. As a result of sequencing, it was found that one clone had only one change of amino acid residue (Asp to Gly change at 81st amino acid residue in SEQ ID NO: 8). This plasmid was named as pMK1130 #3334 and used for further study. Then, the insert fragment of pMK1130 #3334 was cloned into pQE30. This plasmid was named as pMK1209 #3334. After the transformation of expression host, M15 (pREP4), expression study was conducted. M15 (pREP4) (pMK1209 #3334) strain and vector control strain (M15 (pREP4) (pQE30)) were inoculated into 3 ml of LB medium containing 100 µg/ml of ampicillin. After the cultivation at 37 °C for 3.75 hours, cultured broth were divided into two portions. 1 mM IPTG were added to one portion and an incubation was continued for 3 hours. Cells were harvested from 50 µl of broth by the centrifugation and were subjected to SDS-PAGE analysis. Protein which had an expected molecular weight of 48.5 kDa was induced by the addition of IPTG in the culture of M15 (pREP4) (pMK1209 #3334) though no induced protein band was observed in the vector control culture (Fig. 2). This result suggested that activated form of the mevalonate kinase protein could be expressed by the artificial addition of one nucleotide at amino terminal end.

Example 12 Cloning of the mevalonate pyrophosphate decarboxylase (*mpd*) gene

[0090] A cloning protocol of *mpd* gene was almost the same as the *hmc* gene shown in Example 2 to 7. At first, the PCR primers whose sequence were shown in TABLE 14 based on the common sequences of mevalonate pyrophosphate decarboxylase genes from other species were synthesized.

TABLE 14
Sequence of primers used in the cloning of *mpd* gene

Mpd1 ; HTNAARTAYTTGGGNAARMGNGA (sense primer) (SEQ ID NO: 34)
Mpd2 ; GCRTTNGGNCCNGCRTCRAANGTRTANGC (antisense primer) (SEQ ID NO: 35)

(N=A, C, G or T; R=A or G, Y=C or T, H=A, T or C, M=A or C)

[0091] After the PCR reaction of 25 cycles of 95 °C for 30 seconds, 50 °C for 30 seconds and 72°C for 15 seconds by using ExTaq as a DNA polymerase, reaction mixture was subjected to agarose gel electrophoresis. A 0.9 kb of PCR band whose length was expected to contain a partial *mpd* gene was recovered and purified by QIAquick according to the method prepared by the manufacturer and then ligated to pMOSBlue T-vector. After a transformation of competent *E. coli* DH5α cells, 6 white colonies were selected and plasmids were isolated. Two of 6 clones had an expected length of insert. As a result of sequencing, it was found that one of the clones had a sequence whose deduced amino acid sequence was similar to known mevalonate pyrophosphate decarboxylase genes. This cDNA clone was designated as pMPD129 and used for further study.

[0092] Next, a partial genomic fragment which contained *mpd* gene was cloned by PCR. As a result of PCR whose condition was the same as that of the cloning of a partial cDNA fragment the amplified 1.05 kb fragment was obtained and was cloned into pMOSBlue T-vector. As a result of sequencing, it was confirmed that a genomic fragment containing *mpd* gene which had typical intron structures have been obtained and this genomic clone was named as pMPD220.

[0093] Southern blot hybridization study was performed to clone a genomic fragment which contained the entire *mpd* gene from *P. rhodozyma*. Probe was prepared by labeling a template DNA, pMPD220 digested by *Kpn*I, with DIG multiprimer method. Hybridization was performed with the method specified by the manufacturer. As a result, the probe hybridized to a band that had 7.5 kb in its lengths. Next, a genomic library consisting of from 6.5 to 9.0 kb of *Eco*RI fragment in the λZAPII vector was constructed. The packaged extract was infected to *E. coli* XL1Blue, MRF' strain and overlaid with NZY medium poured onto LB agar medium. About 6000 plaques were screened by using the 0.6 kb fragment of *Kpn*I- digested pMPD220 as a probe. 4 plaques were hybridized to the labeled probe. Then a phage lysate was prepared according to the method specified by the manufacturer (Stratagene) and *in vivo* excision was performed by using *E. coli* XL1Blue MRF' and SOLR strains. Each 3 white colonies derived from 4 positive plaques were selected and plasmids were isolated from those selected transformants. Then, isolated plasmids were subjected to a colony PCR method whose protocol was the same as that in example 8. PCR primers whose sequences were shown in TABLE 14, depending on the sequence found in pMPD129 were synthesized and used for a colony PCR.

TABLE 15
Sequence of primers used in the colony PCR to clone a genomic *mpd* clone

Mpd7 ; CCGAACTCTGCTCATCGCC (sense primer) (SEQ ID NO: 36)

TABLE 15 (continued)

Sequence of primers used in the colony PCR to clone a genomic *mpd* cloneMpd8 ; CAGATCAGCGCGTGGAGTGA (antisense primer) (SEQ ID NO: 37)

5 [0094] PCR condition was almost the same as the cloning of *mvk* gene; 25 cycles of 30 seconds at 94 °C, 30 seconds at 50 °C and 10 seconds at 72 °C. All the clone except one produced a positive 0.2kb PCR band. A plasmid was prepared from one of the positive clones and the plasmid was named as pMPD701 and about 3 kb of sequence thereof was determined by the primer walking procedure (SEQ ID NO: 4). There existed an ORF consisted of 402 amino acids (SEQ ID NO: 9) whose sequence was similar to the sequences of known mevalonate pyrophosphate decarboxylase (52.3 % identity to mevalonate pyrophosphate decarboxylase from *Schizosaccharomyces pombe*). Also determined was a 0.4 kb of 5'-adjacent region which was expected to include its promoter sequence.

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Example 13 Cloning of farnesyl pyrophosphate synthase (*fps*) gene

15 [0095] A cloning protocol of *fps* gene was almost the same as the *hmc* gene shown in Example 2 to 7. At first, the PCR primers whose sequence were shown in TABLE 16 based on the common sequences of farnesyl pyrophosphate synthase genes from other species were synthesized.

20 TABLE 16

Sequence of primers used in the cloning of *fps* geneFps1 ; CARGCNTAYTTYTNGTNGCNGAYGA (sense primer) (SEQ ID NO: 38)Fps2 ; CAYTTTRTRTCYTGDATRTCNGTNCCDATYTT (antisense primer) (SEQ ID NO: 39)

(N=A, C, G or T; R=A or G, Y=C or T, D=A, G or T)

30 [0096] After the PCR reaction of 25 cycles of 95 °C for 30 seconds, 54 °C for 30 seconds and 72°C for 30 seconds by using ExTaq as a DNA polymerase, a reaction mixture was applied to agarose gel electrophoresis. A PCR band that has a desired length (0.5 kb) was recovered and purified by QIAquick according to the method prepared by the manufacturer and then ligated to pUC57 vector. After a transformation of competent *E. coli* DH5 α cells, 6 white colonies were selected and plasmids were then isolated. One of the plasmids which had desired length of an insert fragment was sequenced. As a result, it was found that this clone had a sequence whose deduced amino acid sequence was similar to known farnesyl pyrophosphate synthase genes. This cDNA clone was named as pFPS107 and used for further study.

35 [0097] Next, a genomic fragment was cloned by PCR by using the same primer set of Fps1 and Fps2. The same PCR condition as the case of cloning of a partial cDNA was used. A 1.0 kb band yielded was cloned and sequenced. This clone contained the same sequence with the pFPS107 and some typical intron fragments. This plasmid was named as pFPS113 and used for a further experiment.

40 [0098] Then, also cloned was a 5'- and 3'- adjacent region containing *fps* gene with the method described in Example 8. At first, the PCR primers whose sequences were shown in TABLE 17 were synthesized.

TABLE 17

45 Sequences of primers used for a cloning of adjacent region of *fps* geneFps7 ; ATCCTCATCCCGATGGGTGAATACT (sense for downstream cloning) (SEQ ID NO: 40)Fps9 ; AGGAGCGGTCAACAGATCGATGAGC (antisense for upstream cloning) (SEQ ID NO: 41)

50 [0099] Amplified PCR bands were isolated and cloned into pMOSBlue T-vector. As a result of sequencing, it was found that the 5'-adjacent region that had 2.5 kb in its length and 3'-adjacent region that had 2.0 kb in its length were cloned. These plasmids were named as pFPSSTu117 and pFPSSTd117, respectively. After sequencing of both plasmids, it was found that an ORF that consisted of 1068 basepairs with 8 introns. Deduced amino acid sequence showed an extensive homology to the known farnesyl pyrophosphate synthase from other species. Based on the sequence determined, two PCR primers were synthesized with the sequences shown in TABLE 17 to clone a genomic *fps* clone and cDNA clone for *fps* gene expression in *E. coli*.

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TABLE 18

Sequences of primers used for a cDNA and genomic *fps* cloning

5	Fps27 : GAATTCATATGTCCACTACGCCTGA (sense primer) (SEQ ID NO: 42)
	Fps28 : GTCGACGGTACCTATCACTCCCCGCC (antisense primer) (SEQ ID NO: 43)

10 [0100] PCR condition was as follows; 25 cycles of 30 seconds at 94 °C, 30 seconds at 50 °C and 30 seconds at 72 °C. One cDNA clone that had a correct sequence was selected as a result of sequencing analysis of clones obtained by PCR and was named as pFPS113. Next, Southern blot hybridization study was performed to clone a genomic fragment which contained the entire *fps* gene from *P. rhodozyma*. Probe was prepared by labeling a template DNA, pFPS113 with DIG multiprimer method. As a result, labeled probe hybridized to a band that had about 10 kb in its length.

15 [0101] Next, a genomic library consisting of 9 to 15 kb of *Eco*RI fragment was constructed in a λDASHII vector. The packaged extract was infected to *E. coli* XL1 Blue, MRA(P2) strain (Stratagene) and over-laid with NZY medium poured onto LB agar medium. About 10000 plaques were screened by using the 0.6 kb fragment of *Sac*I-digested pFPS113 as a probe. Eight plaques were hybridized to the labeled probe. Then a phage lysate was prepared according to the 20 method specified by the manufacturer (Promega). All the plaques were subjected to a plaque PCR using Fps27 and Fps28 primers. Template DNA for a plaque PCR was prepared by heating 2 μl of solution of phage particles for 5 minutes at 99 °C prior to a PCR reaction. PCR condition is the same as that of pFPS113 cloning hereinbefore. All the plaques gave a 2 kb of positive PCR band, and this suggested that these clones had an entire region containing *fps* gene. One of the λDNA that harbored *fps* gene was digested with *Eco*RI to isolate 10 kb of *Eco*RI fragment and to clone 25 in *Eco*RI-digested and CIAP-treated pBluescriptII KS- (Stratagene). Twelve white colonies from transformed *E. coli* DH5α cells were selected and plasmids were prepared from these clones and subjected to colony PCR by using the same primer sets of Fps27 and Fps28 and the same PCR condition. Two kb of positive band were yielded from 3 of 12 candidates. One clone was cloned and named as pFPS603. It was confirmed that sequence of *fps* gene which was previously determined from the sequence of pFPSSTu117 and pFPSSStd117 were almost correct although they had some 30 PCR errors. Finally, it was determined the nucleotide sequence of 4092 base pairs which contains *fps* gene from *P. rhodozyma* (Fig. 3), and an ORF which consisted of 365 amino acids with 8 introns was found (SEQ ID NO: 5). Deduced amino acid sequence (SEQ ID NO: 10) showed an extensive homology to known FPP synthase (65 % identity to FPP synthase from *Kluyveromyces lactis*).

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SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

(i) APPLICANT: F.HOFFMANN-LA ROCHE. AG

(ii) TITLE OF INVENTION: Improvement of microbiological
carotenoid production and biological materials therefor

10 (iii) NUMBER OF SEQUENCES: 43

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE:
(B) STREET: Grezacherstrasse 124
(C) CITY: BASLE
15 (E) COUNTRY: SWITZERLAND
(F) ZIP: CH-4002

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
20 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

25 (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 061-688 25 11
(B) TELEFAX: 061-688 13 95
(C) TELEX: 962292/965542 hlr c

30 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6370 base pairs
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

40 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Phaffia rhodozyma
(B) STRAIN: ATCC96594

45 (ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 1441..1466

(ix) FEATURE:

(A) NAME/KEY: intron
(B) LOCATION: 1467..1722

50 (ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 1723..1813

5
10
15
20
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30
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45
50
55

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 1814..1914

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 1915..2535

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 2536..2621

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 2622..2867

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 2868..2942

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 2943..3897

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 3898..4030

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(A) NAME/KEY: exon
(B) LOCATION: 4031..4516

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(A) NAME/KEY: intron
(B) LOCATION: 4517..4616

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(B) LOCATION: 4910..5007

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(B) LOCATION: 5082..5195

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(B) LOCATION: 5196..5446

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 5447..5523

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 5524..5756

(ix) FEATURE:
(A) NAME/KEY: polyA_site

(B) LOCATION: 6173

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5	GGAAGACATG ATGGTGTGGG TGTGAGTATG ACCGTGAGCG TGCGTATGGG CCTGGGTGTG	60
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	AGATGTTTC TCTGGTAGG AGCGTTCTGC ATTGGGGCAG GAGAAAAAAAT AGTGTGGTTA	180
10	CGGGAGATCG TGGTTACATC AAGCCATCGT CACTGTAAGG CTCTGTAAGG CTCGGTTGTT	240
	AAGAAGGTAA CCAAGTGTAA TCACTTGGTT CGCGGGGTGA CACTTAGGCT CTGGCGATTA	300
	ATATATCTGA ACCAGACCAA ACTATTAACA ATATACTTT GGATAAGAGG TTTCAACAAAG	360
15	AATCTCAGCT TGAGGAAAAC TCTTATCCAA GAAGGCGCGA GGGCGTCCCC GTTTTATATC	420
	AGGACCCCTC CGCGATTGG TCTGCCACTA AAGATATACA TATGACGAGC CTAGAGAGGC	480
	TCGAGATCAC GAAAACTAAA AAGATGAAGC ATGAACCATG CAAACTAGAG CATGATGAA	540
20	AATGGGCGAA GAGGCATAAG GGATGGAGGG AACGAATAGC CTGTAGGGGT AACCCACGTA	600
	AGAGAACACG TGATACTTAA CCCGTATCCC TGACAGTCAC GGTGTTCTT GAGAGTCAGT	660
	AATGTCCAGC TGTGACCTCA CGTGACTAAA CCCGACACGT GTGCTTCGAC CGAGGTGGGA	720
25	CGATCTTTT TTTGGGGGGA GAAACCGAGT GGGACGATAG AGAGGACTAC GGAGAACTGT	780
	AGTGAATTGT AGTGCCTCA CTACGGAGAG TTCTAGTTGA GCAAGCGATG TGATTTCAA	840
	TACAATCCCG GACTACAAGC TCTCTAAAG AGCTCTATAA TAGAAGGACA AAAGTCGTCC	900
	CACTCCTATC TCCCCGCGGT TTTAATAGAG ACCGATTGTT TTTTCCCTA ATGTTTATT	960
30	TTCTTCCCC GATCGGCTCA TTTTCTCTCT CTCCCGTAT TCTTCACACCA ACGCTCCCTC	1020
	CGATCTTTT TCTTCTTGTGTT CCTGTTCTC TTCTCTCTC TCCATTGTCT TCTTCTCTC	1080
	CTTCCTTCTC TCTTGCCTCT AGCCAGCTTC AACAGCGACG TCTCTCTCTC TCTGTGTGGT	1140
35	GATCTCCGAC TGTAGTGTCT CTCTCGTCA CTTTCACGAA TCAACTTCGT TTCTTTCTG	1200
	ATCGATCGGT CGTCTTCCC TCAATCCGT CATAACTCA CACTTACACT CACACCCACA	1260
	CACTAAACA CGCTAAATAA TCAGATCCGT CTCCCCCTCT TGATCTCCTT CGGCTTAGGC	1320
40	AATGGCTTCC TTGTTCGGCC TCCGGCGGTCT CTCAAACGAG CAGCCGCGCT CTCCCTCTGCT	1380
	CATCCAATCG AAGTCATCCT TTCTACCTTT GTCGTGGTCA CCTTGACGTA CTTTCAGTTG	1440
	ATGTACACCA TCAAGCACAG TAATTTGTAC GTCCGATCAT CTATTGTGCG TGTCTCCTT	1500
45	AGTCTCTTTC TCTTCCTCTT TTGTCCTTCG CGTCAGCGTG GCTGGATTTC CGTCTCCATG	1560
	TCATTTCCCT TATTTCCCTCT TCCTGTCATT TGTTCTCTA CTTTCTTTTC TCTACCTCCT	1620
	TTCCCTGTGCG TTTGCTTCC TTCGCCAGTT GACCACCGAT CCTCAGGATT CATGGCTAAC	1680
50	ATGCCCAACA CAAACTTGCA TATCATCTCT CTTCGTCCAC AGTCTTCTC AGACGATTAG	1740
	CACACAATCT ACCACCAGCT GGGTCGTGCA TGCCTCTTC TCTTTGGGAT CCAGATACCT	1800
	TGACCTCGCG AAGGTTAGTC AGTTGACCCCT CTCATGCTTC TTTTCTCTCA GTCTTGTGTG	1860

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	TGCGCATATA CCCACTCATA GACATCTCG TACGCTGCAC TTTCCCTCCC TTAGCAAGCA	1920
5	GACTCGGCCG ATATCTTAT GGTCCTCCTC GGTTACGTCC TTATGCACGG CACATTGTC	1980
	CGACTGTTC TCAACTTTCG TCGGATGGGC GCAAACTTTT GGCTGCCAGG CATGGTTCTT	2040
	GTCTCGTCCT CCTTTGCCCT CCTCACCGCC CTCCCTGCCG CCTCGATCCT CAACGTTCCG	2100
10	ATCGACCCGA TCTGTCTCTC GGAAGCACTT CCCTTCCTCG TGCTCACCGT CGGATTTGAC	2160
	AAGGACTTTA CCCTCGCAAA ATCTGTGTT AGCTCCCCAG AAATCGCACC CGTCATGTT	2220
	AGACGAAAGC CGGTGATCCA ACCAGGAGAT GACGACGATC TCGAACAGGA CGAGCACAGC	2280
	AGAGTGGCCG CCAACAAGGT TGACATTAG TGGGCCCCCTC CGGTGCCGC CGTCCGTATC	2340
15	GTCATTGGCT CGGTCGAGAA GATCGGGTCC TCGATCGTCA GAGACTTTGC CCTCGAGGTC	2400
	GCCGTCCTCC TTCTCGGAGC CGCCAGCGGG CTCGGCGGAC TCAAGGAGTT TTGTAAGCTC	2460
	GCCGCGTTAA TTTTGGTGGC CGACTGCTGC TTCACCTTTA CCTTCTATGT CGCCATCCTC	2520
20	ACCGTCATGG TCGAGGTAAG CCTTTCTTC AAGTTTCTTG CTGTCATTTT CCTTTCGACA	2580
	CGTATGCTCA TCTTTCTGTTT CCGTCTCTCT CACCTTCCA GGTTCACCGA ATCAAGATCA	2640
	TCCGGGCTT CCGACCGGCC CACAATAACC AACACCCGAA TACTGTGCC TCTACCCCTA	2700
	CTATCGACGG TCAATCTACC AACAGATCCG GCATCTCGTC AGGGCCTCCG GCCCGACCGA	2760
25	CCGTGCCCCGT GTGGAAGAAA GTCTGGAGGA AGCTCATGGG CCCAGAGATC GATTGGCGT	2820
	CCGAAGCTGA GGCTCGAAAC CGGGTTCCAA AGTTGAAGTT GCTCTTAGTA AGTAAACTTC	2880
	CTTTGTTCTT CTCATCATTC TTTATCTCCG AACCTGACG TCGGACCCCTT CTCGATTCAA	2940
30	AGATCTGGC CTTTCTTATC CTTCATATCC TCAACCTTTG CACGCCTCTG ACCGAGACCA	3000
	CAGCTATCAA GCGATCGTCT AGCATAACACC AGCCCATTTA TGCCGACCCCT GCTCATCCGA	3060
	TCGCACAGAC AAACACGACG CTCCATCGGG CGCACAGCCT AGTCATCTTT GATCAGTTCC	3120
35	TTAGTGACTG GACGACCATC GTCGGAGATC CAATCATGAG CAAGTGGATC ATCATCACCC	3180
	TGGGGGTGTC CATCTGCTG AACGGGTTCC TCCCTAAAGG GATCGCTTCT GGCTCTGCTC	3240
	TCGGACCCGG TCGTGCCGGA GGAGGAGGAG CTGCCGCCGC CGCCGCCGTC TTGCTCGAG	3300
40	CGTGGGAAAT CGTCGATTGG AACATGAGA CAGAGACCTC AACGAACACT CCGGCTGGTC	3360
	CACCCGGCCA CAAGAACCG AATGTCACC TCCGACTCAG TCTCGAGCGG GATACTGGTC	3420
	TCCTCCGTTA CCAGCGTGAG CAGGCCCTACC AGGCCAGTC TCAGATCCTC GCTCTTATTT	3480
45	CACCGGTCTC TGTCGCGCCC GTCGTCTCCA ACGGTAACGG TAACGCATCG AAATCGATTG	3540
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	CTTCGACGAC AGAATCAACC CGGGTCAACA AGGTTATCAT CGGTGGACCG TCCGACAGGC	3660
	CTGCCCTAGA CGGACTCGCC AATGGAAACG GTGCCGTCCC CCTTGACAAA CAAACTGTGC	3720
50	TTGGCATGAG GTCGATCGAA GAATGCGAAG AAATTATGAA GAGTGGTCTC GGGCCTTACT	3780
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	TGGAAAAAGC ATTGCAGAAC TGTGAGCGGG CGGTCAAGAT TCGAAGGGCG GTTATCTGTA	3900
5	GGTCCTTTTC TCCTTTGAAT TTCAAGCCTT GGAGGGAGGG AAAGTGCTTC GGGGTACAAT	3960
	ACAGGTTGTG CAAACAAAACC AAGAGAAAACT AAAGAAAACT TTCTCTCCCT CTCTCTCCCC	4020
	TCGACGTCAG CCCGAGCATC CGTTACTAAG ACGCTGGAAA CCTCGGACTT GCCCATGAAG	4080
10	GATTACGACT ACTCGAAAAGT GATGGGCGCA TGCTGTGAGA ACGTTGTCGG ATATATGCCT	4140
	CTCCCTGTG GAATCGCTGG TCCACTTAAC ATTGATGGCG AGGTCGTCCC CATCCCGATG	4200
	GCCACCACCG AGGAACTCT CGTGGCCTCG ACGTCGAGAG GTTGCAAAGC GCTCAACGCG	4260
	GGTGGCGGAG TGACCACCGT CATCACCCAG GATGCGATGA CGAGAGGACC GGTGGTGGAT	4320
15	TTCCCTTCGG TCTCTCAGGC CGCACAGGCC AAACGATGGT TGGATTGGT CGAAGGAATG	4380
	GAGGTTATGG CCGCTTCGTT CAACTCGACT TCTAGATTG CCAGGTTGCA GAGCATCAAG	4440
	TGTGGAATGG CCGGCCGATC GCTATACATC CGTTGGCGA CCAGTACCGG AGATGCGATG	4500
20	GGAAATGAACA TGGCTGGTGA GTGCGACGAG TTTTCTTGT TCTCTTGTG CGGACCATGT	4560
	TTTCTCATCC AGCCAATTCA TTCTTCATTC CTTCTCGGTG TTTGGCAACC TTTTAGGTAA	4620
	AGGAACCGGAG AAAGCTTTGG AAACCCCTGTC CGAGTACTTC CCATCCATGC AGATCCTTGC	4680
	TCTTCTGGT AACTACTGTA TCGACAAGAA GCCTTCTGCC ATCAACTGGA TTGAGGGCCG	4740
25	TGGAAAGTCC GTGGTGGCCG AGTCGGTGT CCCTGGAGCG ATCGTCAAGT CTGTCCTCAA	4800
	GACAACGGTT CGGGATCTCG TCAACTGAA CATTAAAGAA AACTTGATCG GAAAGTGCCAT	4860
	GGCAGGCAGC ATTGGAGGAT TCAACGCCA CGCGTCAAT ATTTGACTG TGCGTACTTC	4920
30	TCTTCCATA TTCGTCCTCG TTTAATTCT TTTCTGTCCA GTCTTATGAC GTCTGATTGG	4980
	TTCTTCTTTT CACCCACACA CATAAGTCATC ATCTTCTTGG CTACAGGTCA GGATCCTGCA	5040
	CAGAAATGTGG AGTCCTCAAT GTGCATGACA TTGATGGAGG CGTACGTTTT TTGTTTGTGTT	5100
35	TTCCCTCTTT TTCCATATGT TTCTACTTCT ACTTTCTTCC CGAGTCCGCC AAGCTGATAC	5160
	CTTTATACGG TCCCTCTCT TCTCATGACG AGTAGTGTGA ACGACGGAAA AGATCTACTC	5220
	ATCACCTGCT CGATGCCGGC GATCGAGTGC GGAACGGTCG GTGGAGGAAC TTTCCCTCCCT	5280
40	CCGAAAAACG CCTGTTTGCA GATGCTCGGT GTGCAGGTG CCCATCCAGA TTGCCCCGGT	5340
	CACAATGCTC GTCGACTAGC AAGAACATC GCTGCCAGTG TGATGGCTGG AGAGTTGAGT	5400
	TTGATGAGTG TTTGGCCGC TGTCATTTA ATCAAGGCC ACATGAGTAA GTCTGCCACC	5460
	TTTGATAAT CAAAAGGGTC GTGGTACTGG TGTCACTGAC TGTTGACTCT TCCGTGTCATG	5520
45	CAGAGCACAA TCGATCGACA CCTTCGACTC CTCTACCGGT CTCACCGTTG GCGACCCGAC	5580
	CGAACACGCC GTCCCACCGG TCGATTGGAT TGCTCACACC GATGACGTCT TCCGCATCGG	5640
	TCGCCTCGAT GTTCTCTGGG TTGGTAGTC CGTCGACGAG CTCGCTCAAG ACGGTAGGTA	5700
50	GCATGGCTTG CGTCAGGGAA CGAGGGAGC AGACGAGTGT GAAACGTGGAT GCCTGAACGT	5760
	GGGACTCCCT TTTCTTGGTA TCCCCTCCGT TTTCTTTCG GCCTTGAAT CCTGTATTCT	5820

	TGTCCGTTTT TTCATCTTCT CTTCCCTGGTT CTCCCTCTCT CGTCATCTG CAAAAACAAA	5880
	ATTCAATCGC ATCGGTCTCT GGCATTCCAT TTGGGTTTCA AAATCAAATC AATCTCTATC	5940
5	TACTATCTCA AATATCTTTT TTTCATCTTT TGATTCAATT CTGTTGAAAA CTGTCCTGCC	6000
	CTTCTCCTAC TTCTTATCTC TGCCCTCTTG CCAAAGTCA ATTCTGTGTC CATCTGTGCA	6060
	CTCTGATCTA TCAGTCTGTA TCAAGTACGC TCTTAAATCT GTAATTGGCT CTCGGAGGTG	6120
10	TCTCGTCATC TCACATATGG CTGGCGATAT GATGTGTCGG TTTCTTCCCC TCCAACAAAG	6180
	GCGACGTGGC TCCTTCATCA ATCTTGGCG CAAGCTCTCA AAATCTCCA AAACGGCTGA	6240
	CTAAGCAAGG TTCCAAGTA CTCTCAAACC GAGCAAGGCC ATCCATCTC AAATCAACTT	6300
15	GTGAAACCCCT TTGTGGATAG ACCGTCCAAA CCGAGCTCTT CCCAATCTTC GCCTCCCCTT	6360
	CTTCCTGCAG	6370

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4775 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Phaffia rhodozyma
 - (B) STRAIN: ATCC96594
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1305..1361
- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 1362..1504
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1505..1522
- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 1523..1699
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1700..1826
- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 1827..1920
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1921..2277

5 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2278..2351

10 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2352..2409

15 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2410..2497

20 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2498..2504

25 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2505..2586

30 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2587..2768

35 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2769..2851

40 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2852..2891

45 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2892..2985

50 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2986..3240

55 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 3241..3325

60 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 3326..3493

65 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 3494..3601

70 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 3602..3768

75 (ix) FEATURE:
 (A) NAME/KEY: polyA_site
 (B) LOCATION: 4043

80 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CATCGAACAG AGCGAAAGTGA TTAGGGAAGC CGAACAGGGCA CTAACAAACGT GGTTGTATAT
GTGTGTTTAT GAGTGTTATA TCGTCAGGAA CGAACGTCAT TCATTTAGCT AGACAGGGAG

	AGAGGGAGAA ACCTACGGGT TTACCCATT GGACCAGTCT AAAGAGAGAA CGAGAGTTTT	180
5	TGGTCGGTC ACCTGAAGAG TTTAACCTC CACAAGTTA TTCTAGATTA TTTCCGGGG	240
	TATGTGAAGG ATAATGTCAA ACTTTGTCCA GATTGAAGAA GGCAAGAAAG GAAAGGGCG	300
	AACGAGAGTA TCGCCCCATC TATGGGTGAC CAGTCGACCT TCTGCATCGG CGATCCCAG	360
10	AATGGAAGGT TCCGATGGAT CAGAAGTAGG TTTCTAAGC TCAACATAG GTCATTGCGA	420
	GTGAGATACA TATGCAGACT GATATGCTAG TCAAACCGAA CGAGATTCT CTGTTTGCTT	480
	TCAAAAAGAC GAACCAACCA TTTCATGTCC AAGATGGCAG GTCCCTCGAT TCTTGAAAGC	540
	TCCTCCCTGA TGCGGACAGA AAAGAATAAA AAGTAGACAG ACTGTCAAGT CGACAGGCCA	600
15	AGTTTATCAA GCTGAGCAG AAAACTCGAA CTPACATACC TTGGCCGTCA GTTCTGTAGA	660
	CCAAGCATCG GCCTTCCTC TTTGCGCAG GTGTACCGT TGGCTCACCA TCGTCACTCT	720
	CGTCTCTGA CCCGTTGCTT TCCTTGACAG CAGTCTGTC CACAGGTTTC TCTAACTGAT	780
20	AGGTCCAAAC AGCAAAGATA TCTGGATGTC TATGTGAGAA CTCTACTGAG TCGGCAGAGT	840
	ACACCGTATC GATATAGGCG AGTGAGGAAG CTTTGAAGG TGAAGAAGTA GCGAAAGATC	900
	ATCAGCGAAT GAGGACTATG ACAAAAAAGA AATTTTCGTA TAATCCACTG GACAAATCAC	960
25	CTTCCATCGT GTCCCTCAAG AGGGTTTCGT CTGAAACGTA AGGACGAGGT ATTGATAGAT	1020
	GATTGACCTT GAGTACCGGG ATGGACAAGG AACGAGCCCA CTCCCAGGGC TATGTAACAC	1080
	CACACGTGAC TCCACTTGAA TTGCGCAGA TAAACGAAGT CTTACGATCG GACGACTTGT	1140
	TAACCATTAA GTTATTACCG CGTCTGTGTT TCCTACTTTC ATCGTCCAT TTTAGACACA	1200
30	AAAAAAAGAAG CCAGAAGAGA AAAGAATAAA ACGTCTACCG TGTTCTCTCC GAATTCTTAC	1260
	CACACCCACA AAACCATAACA CAATCTCAAT CTAGATATCC AGTTATGTAC ACTTCTACTA	1320
	CCGAACAGCG ACCCAAAGAT GTTGAATTTC TCGGTATGGA GGTATGTTGT TCAATTCTGT	1380
35	TTGTGTTCAA TCTTTAATCA TCTTTAGTCG ACTGACCGGT TCTTCCTTTT TTTTCTTC	1440
	TCAAACAAA CAACCCCTCT CGATTCTATGT CATCTTCTT TCCAATGCGC TACTCCTCT	1500
	GTAGATCTAC TTTCTCGAC GAGTGCCTAA CTATTCTCTC TTCTGCATTC TCTCTCTATT	1560
40	CCCATGTTG ATCCCTCGCC CTCATATGGG CGACTGTTTC ATCTCTTTG CTTCGTCCA	1620
	TTCTCTTTG ATCTGTTCA TTTCTACTA ATATCTCCG ACGCGAAATA CAACACTGAC	1680
	CGCGATTCTCT CTCGATCAGG CCATCGCTCA CAAGGATCTC GAGGCTTTG ATGGGGTTCC	1740
45	TTCCGGAAAG TACACCATCG GTCTCGGCAA CAACTTCATG GCCTTCACCG ACGACACTGA	1800
	GGACATCAAC TCGTTCGCCT TGAACGGTCA GTCTCTCCG TTTCAGCAAT CGACAGGAAA	1860
	AAGGCCCAAG CGCATCTCAC TGACACCTTT CTCCGTTTG CAATCCATT TGATTGTTAG	1920
50	CTGTTCCGG TCTTCTATCA AAGTACAACG TTGATCCAA GTCAATCGGT CGAATTGATG	1980
	TCGGAACTGA GTCCATCATT GACAAGTCCA AATCTGTCAA GACAGTCCTT ATGGACTTGT	2040
	TCGAGTCCCA CGGCAACACA GATATTGAGG GTATCGACTC CAAGAATGCC TGCTACGGTT	2100

	CTACCGCGGC CCTGTTCAAT GCCGTCAACT GGATCGAGTC ATCCTCTGG GACGGAAGAA	2160
5	ATGCCATTGT CTTCTGCGGA GACATTGCCA TCTACGCCA GGGTGCTGCC CGACCTGCCG	2220
	GAGGTGCTGG TGCTTGCCTG ATCCTCATCG GACCCGACGC TCCCCTGCTG TTCGAGCGTG	2280
	AGTTCCAATC CGTCATTTTC TTCCACGGCA GCGGCTGAAA CAACCTTAT CCGTCATTCT	2340
10	CATCAATCTA GCCGTCCACG GAAACTTCAT GACCAACGCT TGGGACTTCT ACAAGCCTAA	2400
	TCTTTCTTCG TATGTTCAAA TTTTGAAGTT TGCGCTTGGG AGAGTCTTAC ACTAATTCTGG	2460
	GGTGCTCGTA CCCTTCAAT CGTTTGTGCTT TTTATAGTGA ATACGTTCTG CTGCGCACCT	2520
	CCTATATTTA GTTTTGATC AAATATTGTC CATTGAATTA ACTCTGAAAC CTTCTCCTCC	2580
15	AAATAGCCCCA TTGTCGATGG ACCTCTCTCC GTCACTTCCT ACGTCAACGC CATTGACAAG	2640
	GCCTATGAAG CTTACCGAAC AAAGTATGCC AAGCGATTTG GAGGACCCAA GACTAACGGT	2700
	GTCACCAACG GACACACCAGA GGTTGCCGGT GTCAGTGCTG CGTCGTTGCA TTACCTTTTG	2760
20	TTCCACAGGT AAGCGTCATC TTCTGTATTTC TCCTTAAATT CAACCGATCA ACGGAGTTAA	2820
	TTCGTGTCA CATATTATCT TGTTGGAAACA GTCCTTACGG AAAGCAGGTT GTCAAAGGCC	2880
	ACGGCCGACT TGTAAGCAGT CTTTTGTAA CTCTTAGCTT GCAGATAAAA ACTTTTAGGT	2940
	TTCTGGTACT CATTATTTAT GCATCTCTG AATCACCTTA TCTAGTTGTA CAATGACTTC	3000
25	CGAAACAAACC CCAACGACCC GGTTTTGCT GAGGTGCCAG CCGAGCTTGC TACTTTGGAC	3060
	ATGAAGAAAA GTCTTTCAGA CAAGAATGTC GAGAAATCTC TGATTGCTGC CTCCAAGTCT	3120
	TCTTCAACA AGCAGGTTGA GCCTGGAATG ACCACCGTCC GACAGCTCGG AAACCTGTAC	3180
30	ACCGCCTCTC TCTTCGGTGC TCTCGCAAGT TTGTTCTCTA ATGTTCTGG TGACGAGCTC	3240
	GTAAGTCTTG ATCTCTATCC CAATCATCTC TTCCCTTATCA ATTGAACGTA ACTCTTTCT	3300
	TTAATGCTGG CTTTCTCTTG AACAGGTCGG CAAGGCATT GCTCTCTACG CCTACGGATC	3360
35	TGGAGCTGCT GCTCTTTCT ATGCTCTTAA GGTCAAGAGC TCAACCGCTT TCATCTCTGA	3420
	GAAGCTTGAT CTCAACAACC GATTGAGCAA CATGAAGATT GTCCCCGTG TGACTTTGT	3480
	CAAAGCTCTG AAGGTACGTT GGATAATGAC TTTTTTGTC GACCGTGGTC TTTGTCAACC	3540
40	GCTAACAAACC TTCTTGAATC GGTCTCTTT GGTTGAAAT TCGCTCGCG CTTCGACACA	3600
	GGTCCGAGAA GAGACTCACA ACGCCGTGTC ATATTCGCCA ATCGGTTCGC TTGACGATCT	3660
	CTGGCCTGG A TCGTACTACT TGGGAGAGAT TGACAGCATG TGCGTGCAC AGTACAAGCA	3720
	GGTCCCCCTCT GCTTGAACGG GATATTTAAA GTTCAAAAG TTATGAAAGA GGTCGGCGAA	3780
45	GATTCAAAAT AAATAAAAT AACACCTTG TTTTGGCTT GTTTCTCTTC TTCACTCTCG	3840
	TTTCCGATGT GTTTCTCCG TTTCTCCCT CTTTGTGTC TTTTCTCTCC CTCTTTGGT	3900
	TACAATCTCT TTGGGTTTTA CAGGCTGGCA ATCTCTGTAC AATCTTCGTT CGCGTGATCC	3960
50	GACATAGATA CCGTTGTGGC ATACACCTTG CGTCTTACAT CTTTTGAGAG CTTCGGAGGT	4020
	GATCTTGTGATG AAGAAAATTC ACCATTGACT CCCATCTCTT GAATGTCCTG ACTAAATTGA	4080

	ATTGGAAGCA ACTTATATGA AGAGCAAATT GATGGATCCA GAAAGGAACA AGTCTAGAAA	4140
5	TCAGTGATTT GTGCGAAAAA TCAGCAAATG CCGCGCTGAG CCGCTCGCTG GGGAGTAGAC	4200
	ATTGCCCATG CGCGTGTGATGT TGTCTGACCG TTCTCCTCCA TTCCCCCACT CTCAACCTTC	4260
	CTCTCTTGA GAATCGAAGA AGAAGGCGAA GAAAACCTGA CTTGATCCTT TACAGGGTGT	4320
10	TTCTTTGTT CGTATCTGAG TTACTTTCC TCCTTTCCCTT CCTGCTTGAG TGAATGACTG	4380
	ATCTGACTCC TCCGCCTACC TCGGCGACTG GGCTATATCT TGAGGATAGA ATATCCCCCT	4440
	GACAATCCCA TTTCTCAAGA TTCTTCAAA CAAGAAAACT AGTTCCAATC AATAGATCAT	4500
15	CTGATCAACC TTGTGTGAAC ATAATCATCT GCAGAACGAC TGAAC TGAGA AAGTCTTCCT	4560
	CAGAGGAAAG AGAATACTAG ATAAGATCAT TCGGTTGGGA AGGTAAAGGA ATGAAGTCTG	4620
	GTTCTGGGTT TAGCTCTGGT TCCGTAGGGG GTTCGACTAT AGTTCTTCT GTTCGACTAG	4680
	AAACAGGAGA AACCGTACAT GTAAATGGTA TGATATTCTT GTCTCTGTAT CATGTCCCGC	4740
20	TCATCTCTTT GTTGCAAGT CACTCTGGAG AATTC	4775

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4135 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Phaffia rhodozyma*
 - (B) STRAIN: ATCC96594

- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1021..1124

- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 1125..1630

- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1631..1956

- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 1957..2051

- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 2052..2366

- (ix) FEATURE:
 - (A) NAME/KEY: intron

(B) LOCATION: 2367..2446

5 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2447..2651

10 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2652..2732

15 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2733..3188

(ix) FEATURE:
 (A) NAME/KEY: polyA_site
 (B) LOCATION: 3284

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACTGACTCGG CTACCGGAAA ATATTTTC AGGACGCCTT GATCGTTTG GACAACACCA	60
TGATGTCACC ATATCTTCAG CGGCCGTTGG AGCTAGGAGT AGACATTGTA TACGACTCTG	120
GAACAAAGTA TTTGAGTGGG CACCCAGATC TCATGGCTGG TGTGATTACT ACTCGTACTG	180
AGGAGAGTTGG GAAGGTTCCGT GCTTGCTTGC TTTGAATGTC GTGCCTAAAG CCATTGCCAT	240
AAGACAGAGT CTGATCTATG TCGTTTGCT ACAACAGAGA ATGGCCTGGT TCCCAAATGC	300
TATGGAAAT GCATTGTCTC CGTTCGACTC GTTCCCTTCTT CTCCGAGGAC TCAAAACACT	360
TCCTCTCCGA CTGGACAAGC AGCAGGCCTC ATCTCACCTG ATCGCCTCGT ACTTACACAC	420
CCTCGGCTTT CTTGTTCACT ACCCCGGTCT GCCTTCTGAC CCTGGGTACG AACTTCATAA	480
CTCTCAGGCG AGTGGTGCAG GTGCCGTAT GAGCTTGAG ACCGGAGATA TCGCGTTGAG	540
TGAGGCCATC GTGGCGGAA CCCGAGTTTG GGGAAATCAGT GTCAAGTTCG GAGCCGTGAA	600
CAGTTTGATC AGCATGCCTT GTCTAATGAG GTTAGTTCTT ATGCCTTCTT TTGCGGCCTT	660
CTAAAATTTG TGGCTGACTA ATTGGGTCGG TCTTCCGTT CTTGCATTTG AGTCACGCAT	720
CTATTCCCTGC TCACCTTCGA GCCGAGCGAG GTCTCCCCGA ACATCTGATT CGACTGTGTG	780
TCGGTATTGA GGACCCCTCAC GATTTGCTTG ATGATTTGGA GGCCTCTCTT GTGAACGCTG	840
GCGCAATCCG ATCAGTCTCT ACCTCAGATT CATCCCGACC GCTCACTCCT CCTGCCTCTG	900
ATTCTGCCTC GGACATTCAC TCCAATGGG CCGTCGACCG AGCCAGACAG TTGAGCGTG	960
TTAGGCCTTC TAACTCGACA GCCGGCGTCG AAGGACAGCT TGCGAACTC AATGTAGACG	1020
ATGCAGCCAG ATTTGCGGGC GATGAGAGCC AAAAAGAAGA AATTCTTGTC AGTGCACCGG	1080
45 GAAAGGTCAT TCTGTTGGC GAACATGCTG TAGGCCATGG TGGTGTGAGT GAGAAATGAA	1140
AGCTTTATGC TCTCATTGCA TCTTAACCTT TCTCGCCTT TTTTGTCTC TTCATCCCGT	1200
CTTGAMGTA GGGATGCCCG CCTTTGCCCG TTCCCCCTTC TTGCATCTGT CTATATTTCC	1260
50 TTATACATTT CGCTCTTAAG AGCGTCTAGT TGTACCTTAT AACAAACCTTT GGTTTTAGCA	1320
TCCCTTGATT ATTCAATTCTC CTCATCTTC GGTCAAGAGGC TTTCGGCCAT CTTTACGTCT	1380

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	GATTAGATTG TAATAGCAAG AACTATCTTG CTAAGCCTTT TCTCTTCCTC TTCCCTCCTAT	1440
5	ATAAAATCGAA TTCACCTTCG GACATGTTA TTTTGGGAA ATCATCAAGG GGTGGGGGGC	1500
	CAATCCCGAC ACTAATTTTC TGCTCACGTC AAAACTCAGC GTTCAGAAC AGTCACTGAC	1560
	CCTGATACGT GTCTCTATGT GTGTGGGTGT ACGTGCGAAT TGTGACTCGA CGTTCTACGC	1620
10	TTAAAAACAG ACCGGGATCG CTGCTTCCGT TGATCTCGA TGCTACGCTC TTCTCTCAC	1680
	CACTGCTACG ACAACAACAT CATCGTCGTT ATCGTCTACA AACATTACCA TCTCCCTAAC	1740
	GGACCTGAAC TTTACGCAGT CTTGGCCTGT TGATTCTCTT CCTTGGTCAC TTGCGCCTGA	1800
	CTGGACTGAG GCGCTTATTTC CAGAATCTCT CTGCCCGACA TTGCTCGCCG AAATCGAAAG	1860
15	GATCGCTGGT CAAGGTGGAA ACGGAGGAGA AAGGGAGAAG GTGGCAACCA TGGCATTCTT	1920
	GTATTGTTG GTGCTATTGA GCAAAGGGAA GCCAAGGTAG GTTTTTCTG TCTCTCTTT	1980
	TTGCCATATAA AGACTCTAA CTGACGGAGA AAGTGTGGG TTTCTCCTT CGGGGGTTCA	2040
20	ATCAATTAAA GTGAGCCGTT CGAGTTGAGC GCTCGATCTG CGCTTCCGAT GGGAGCTGGT	2100
	CTGGGTTCAT CGCCCGCTCT ATCGACCTCT CTTGCCCTAG TCTTTCTCT CCACCTTTCT	2160
	CACCTCAGTC CAACGACGAC TGGCAGAGAA TCAACAATCC CGACGGCCGA CACAGAAGTA	2220
25	ATTGACAAAT GGGCGTTCTT AGCTGAAAAA GTCATCCATG GAAATCCGAG TGGGATTGAT	2280
	AACCGGGTCA GTACGAGAGG AGGCCTGTT GCTTCAAAA GAAAGATTGA GGGAAACAG	2340
	GAAGGTGGAA TGGAAGCGAT CAAGAGGTAC GCAGACACGG TGCTTCATAT GCCATACTCC	2400
	AGCTGATTG ACCCATGATG AACGTCTTC TACATTCGA ATATAGCTTC ACATCCATTC	2460
30	GATTCTCAT CACAGATTCT CGTATCGGAA GGGATACAAG ATCTCTCGTT GCAGGAGTGA	2520
	ATGCTGACT GATTCAAGGAG CCAGAGGTGA TCGTCCCTTT GTGGAAGCG ATTCAAGCAGA	2580
	TTGCCGATGA GGCTATTGCA TGCTTGAAAG ATTCAAGAGAT GGAACGTGCT GTCATGATCG	2640
35	ATCGACTTCA AGTTAGTTCT TGTTCTTTC AAGACTCTTT GTGACATTGT GTCTTATCCA	2700
	TTCATCTTC TTTTTCTTC CTCTCTCTGC AGAACTTGGT CTCCGAGAAC CACGCACACC	2760
	TAGCAGCACT TGGCGTGTCC CACCCATCCC TCGAAGAGAT TATCCGGATC GGTGCTGATA	2820
40	AGCCTTCGA GCTTCGAACA AAGTTGACAG GCGCCGGTGG AGGTGGTTGC GCTGTAACCC	2880
	TGGTCCCCGA TGGTAAAGTC TCTCCTTTTC TCTTCCGTCC AAGCGACACA TCTGACCGAT	2940
	GCGCATCCTG TACTTTGGT CAACCAGACT TCTCGACTGA AACCCCTCAA GCTCTTATGG	3000
45	AGACCGCTCGT TCAATCATCG TTGCCCCCTT ATATGCCCG AGTGGGTGGT TCAGGGCTCG	3060
	GATTCTCTTC ATCAAATCAAG GCCGATCCGG AAGATGGGGA GAACAGACTT AAAGATGGGC	3120
	TGGTGGGAAC GGAGATTGAT GAGCTAGACA GATGGGCTTT GAAAACGGGT CGTTGGTCTT	3180
50	TTGCTTGAAC GAAAGATAGG AAACGGTGAT TAGGGTACAG ATCCTTTGCT GTCACTTTTA	3240
	CAAAACACTT TCTTATGTCT TCATGACTCA ACGTATGCCC TCATCTCTAT CCATAGACAG	3300
	CACGGTACCT CTCAGGTTTC AATACTGAAG CGTTCATCGA CAAAACATGC GGCACACGAA	3360

	AACGAGTGGAA TATAAGGGAG AAGAGAGATA TTAGAGCGAA AAAGAGAAGA GTGAGAGAGG	3420
	AAAAAAATAA CCGAGAACAA CTTATTCCGG TTTGTTAGAA TCGAAGATCG AGAAATATGA	3480
5	AGTACATAGT ATAAAGTAAA GAAGAGAGGT TTACCTCAGA GGTGTGTACG AAGGTGAGGA	3540
	CAGGTAAGAG GAATAATTGA CTATCGAAAA AAGAGAACTC AACAGAAGCA CTGGGATAAA	3600
	GCCTAGAATG TAAGTCTCAT CGGTCCGCGA TGAAAGAGAA ATTGAAGGAA GAAAAAGCCC	3660
10	CCAGTAAACA ATCCAACCAA CCTCTTGGAC GATTGCGAAA CACACACACG CACGCGGACAA	3720
	TATTTCGTAC ACAAGGACGG GACATTCTTT TTTTATATCC GGGTGGGGAG AGAGAGGGTT	3780
	ATAGAGGATG AATAGCAAGG TTGATGTTTT GTAAAAGGTT GCAGAAAAAG GAAAGTGAGA	3840
15	GTAGGAACAT GCATTAAGAA CCTGCCAAA GCGATTTATA TCGTTCTTCT GTTTTCACTT	3900
	CTTTCCGGGC GCTTCTTAG ACCGCGGTGG TGAAGGGTTA CTCTGCCAA CTAGAAGAAG	3960
	CAACATGAGT CAAGGATTAG ATCATCACGT GTCTCATTTG ACGGGTTGAA AGATATATT	4020
	AGATACTAAC TGCTTCCAC GCCGACTGAA AAGATGAATT GAATCATGTC GAGTGGCAAC	4080
20	GAACGAAAGA ACAAAATAGTA AGAATGAATT ACTAGAAAAG ACAGAATGAC TAGAA	4135

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2767 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Phaffia rhodozyma
 - (B) STRAIN: ATCC96594
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 401..451
- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 452..633
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 634..876
- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 877..1004
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1005..1916
- (ix) FEATURE:

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(A) NAME/KEY: polyA_site
 (B) LOCATION: 2217

5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:	
	GAATTCTTCC CGACTGGGCT GATCGACTTG ACTGGAAGAT CTAAGGCAGA GGGATGAAGG	60
	AAGTAATTGG AGGGAATGAG GAAAAAAAAGA GGCGAGGGAA CGCGGTCTTC TTTCTGGCA	120
10	AGGCAATGTC GTGTATCTCT CTTGATTCTT TCGTTGTATC GACGGACAC ACTCTTTCG	180
	AATGAATATC ACTATCGCAT CCAATGATCG CTATACATGG CATTACATA TGCCAGACAT	240
	CGCTGAGAAA GAGAGAACAT TCCTTGGAA AAAGCCTACT GTGCCTGAAG TCAGGCTGAT	300
15	GTTGATTAAA CGTCTTCCC CATCTAACG AGACAAACAA CTTCTTTCG TTCAACACAC	360
	CACCTCTCTC CGAAAAAGCT CTTCAATCCA GTCCATTAAG ATGGTTCATC TCGCTACTGC	420
	CTCGGCTCCC GTTAACATTG CGTGTATCAA GGTCCGCTCG CATTGTGAAT GCTGCTCGTT	480
20	TGCCTTGTGT GCGTTTGGTG GATCTGAAAG AACCTTGCT TGAACCATTG CATCTCTGCT	540
	CTTTTCTTC CTGTCCTTTC CTTTTCTCA CGACAAAAAA ACCACCTGGA CCCTTTGTGT	600
	TCCTTCCAT TGGTGTTCAT ACACCTAACG CAGTACTGGG GTAAACGGGA TACCAAGTTG	660
	ATTCTCCCTA CAAACTCCTC CTTGCTGTC ACTCTCGACC AGGATCACCT CCGATCGACG	720
25	ACGTCTTCTG CTTGTGACGC CTGCTCGAG AAGGATCGAC TTTGGCTAA CGGGATCGAG	780
	GAGGAGGTCA AGGCTGGTGG TCGGTTGGAT GTCTGCATCA AGGAGATGAA GAAGCTTCGA	840
	GCGCAAGAGG AAGAGAAGGA TGCCGGCTCG GAGAAAGTGA GTTTTCTCC TGTGTGCGTG	900
30	TGTACTCTGT ATAGGTACCG TTGACAGGAC AGTCTTCTG AAGAGTTGG ATCTTACTCT	960
	TTTTTGGGGG GGTGGTGGTG TTTGAAATAA TGACCAAAAT AAAGCTCTCA TCTTTCAACG	1020
	TGCACCTTGC GTCTTACAAC AACTTCCCGA CTGCCGCTGG ACTTGCTTCC TCCGCTTCCG	1080
35	GTCTAGCTGC GTTGGTCGCC TCGCTCGCCT CGCTCTACAA CCTCCCAACG AACGCATCCG	1140
	AACTCTCGCT CATGCCCGA CAAGGTTCTG GTTCTGCCTG CCGATCGCTC TTCGGGGGT	1200
	TCGTTGCTTG GGAACAGGGC AAGCTTCCT CTGGAACCGA CTGCTCGCT GTTCAGGTG	1260
	AGCCCAGGGG ACACTGGCCC TCACTCCACG CGCTGATCTG TGTAGTTCC GACGAGAAAA	1320
40	AGACGACGGC CTCGACGGCA GGCATGCAA CCACGGTGAAC CACCTGCCT TTGCTCAAAC	1380
	ACCGAATCGA ACACGTCGTT CCAGCCCGA TGGAGGCCAT CACCCAGGCG ATCCGGGCCA	1440
	AGGATTCGCA CTCGTTCGCA AAGATCACCA TGAAGGACTC CAACCAGTTC CACGCCGTCT	1500
45	GCCTCGATTG GGAACCCCCG ATCTTTTACT TGAAACGATGT CTCCCGATCG ATCATCCATC	1560
	TGTCACCGA GCTCAACAGA GTGTCGGTCC AGGCCGGCGG TCCCGCTCTT GCCGCCTACA	1620
	CGTTGACGC CGGGCCGAAC GCGGTGATCT ACGCCGAGGA ATCGTCCATG CCGGAGATCA	1680
50	TCAGGTTAAT CGAGCGGTAC TTCCCGTTGG GAACGGCTTT CGAGAAACCG TTCGGGGTTA	1740
	ACACCGAAGG CGGTGATGCC CTGAGGGAAG GCTTTAACCA GAACGTCGCC CCGGTGTTCA	1800
	GGAGGGAAAG CGTCGCCCCGG TTGATTACACA CCCGGATCGG TGATGGACCC AGGACGTATG	1860

	GGCAGGGAGGA GAGCCTGATC GGCGAAGACG GTCTGCCAAA GGTCGTCAAG GCTTAGACTA	1920
5	TAGGTTGTTT CTTCTAAATT TGAGCCTTCC TCCCCTCCTCC CTTCCACAAG CATAAAACAA	1980
	AGGATAAAACA AATGAATTAT CAAAATAACT ATAGGTTGTT TCTTCTAAAT TTGAGCCTTC	2040
	CTCCCGCCTC CCTTCCACAA GCATAAAACA AAGGATAAAC AAATGAATTAA TCAAAATAAA	2100
10	ATAAAAAGTC TGCCCTCTTT GTTTGGAAT ACATCTTCTT TGGGACATGA CCCTTCTCCT	2160
	TCTTTCCGT ATACATCTTT TTGGGTATTT CATGGTGATC AAACAACATT GTGATCGAAA	2220
	GCAGAGACGG CCATGGTGCT GGCTTGAGC GTCTGGCGTT TTGTGTGTCC TGCACTTGAG	2280
15	CAACCCCCAAG CTGACCGCTA GGAAAACCTCA TTGATGTGAT TTATATCGTA CGATGAAAGA	2340
	GAATAAAATG ATAGAAGAAC AAAGAACAAAC AAAGTAGAACG AACGTCTGAG AAGAAAGACA	2400
	GGAAAATGAC ACGTACATAG TGTCGATGA TGAATGATAT AATATTAAAT ATAAAATGAG	2460
20	GTAACACGTAT AGCATCACCG GATGAACCGA TGAACATGTA GTGGACAAGG TTGGAAATA	2520
	GGAATGTAGA ATCCAAGAAT CGTTGACTGA TGGACGGACG TATGTAAACA GGTACACCCC	2580
	AAAGAAAAGA AAGAAAGAAA GAAAGAAAAC ACAAAAGCCAA GGAAGTAAAG CAGATGGTCT	2640
	TCTAAGAATA CGGCTTCAAA AAGACAGTGA ACACTCGTG TCGAGGAATG ACAAGAAAAG	2700
25	TGAGAGACTA CGAAAGGAAG AAACCAAGAC GAAAAGAAGA ACGGAGATCG AACGGACAGA	2760
	AATAAAAG	2767

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4092 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Phaffia rhodozyma
 - (B) STRAIN: ATCC96594
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 852..986
- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 987..1173
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1174..1317
- (ix) FEATURE:
 - (A) NAME/KEY: intron

(B) LOCATION: 1318..1468

5 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 1469..1549

10 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 1550..1671

15 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 1672..1794

(ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 1795..1890

20 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 1891..1979

(ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 1980..2092

25 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2093..2165

(ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2166..2250

30 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2251..2391

(ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2392..2488

35 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2489..2652

(ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2653..2784

40 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2785..2902

(ix) FEATURE:
 (A) NAME/KEY: polyA_site
 (B) LOCATION: 3024

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGCCCGGTAT CTTGCCACAG ATGCCGCCGG AGTGTCTGGC GGAGTGCTAG GAAACAACGTC	60
50 ATCTCCATCT GACCGAGCAAG CGTAGCACAA GCTAGCTCTT CGTCTGTCAG AAGGACATCC	120
ACGCACCTTC CTGGCCTTCG GGGATGGCAC CTTCTGTCG ACTTCCCATG GCCGTGCCCC	180

	TGGCCTTGTG	AAGATACTGT	TTGCCAAGCT	GAGCGCCCTCC	CCGCTGCTCC	AGGTCCGCAA	240
5	GGTCCGAGAG	TATTGGACGT	CGAAGATATG	TTCAAAGTGT	CAGGGAGTT	CTCGGGAGAA	300
	AAAAAAAGCG	TGGGCTCTGA	AACAGTGTGG	AAATGTCTAC	AAAGTGAGCT	GGATTATTG	360
	TGTGTGTATG	TGTGTGTGTG	TGTATGTTCT	GTGTTGGTTG	CTCACTGTAC	TCTATGCTCT	420
10	CTCTTAGATT	TGGGGAACAG	TGCTGTGAAC	GCCTCGCGAA	ACATGCTGCA	CCTAGCCCTT	480
	CACCAAGAAGG	AGAACCCAGAG	GGCGGGAAATG	CTGGTGTCTG	ACGCTGCTAC	TGCTGCTACG	540
	CTAGCCGCTG	AGGCTGAGGC	TGGCAGAAAAC	TAATCCATG	ACCCATCAGA	TCTTGGTGT	600
15	TCGTGGTCTG	AGGACACCCA	AGTCCAAAAG	GGCTATATAT	CGACCATCAT	CCGTTGCGGT	660
	CACTCAGTAG	TAACTAAAGC	TATACATAGG	AATGTTCTGA	ACTTGATAAC	CCTAACACTA	720
	CGAAAATATC	TCGGAAAATA	GATTAATTTC	CTTCTCATCT	CAAACAAAAG	ACACAACACC	780
	ATCAATCACG	CTCCTTCAC	ACACTCTCCT	TTTGCTCTC	TCGTTCGACA	GAAAATAACA	840
20	TCAATAGCCA	AATGTCCACT	ACGCCTGAAG	AGAAGAAAGC	AGCTCGAGCA	AAGTCGAGG	900
	CTGCTTCCC	GGTCATTGCC	GATGAGATTC	TCGATTATAT	GAAGGGTCAA	GGCATGCCTG	960
	CCGAGGCTTT	GGAATGGATG	AACAAGGTTG	GTCAAGGGTT	TCTTCTTTAT	TCTTCTGGTC	1020
25	TTTGTTCGG	TCGAACCTGGC	TTTCGAACCT	GGCCTTGACC	GGTTGGATCT	CGGTTGTTGC	1080
	GCCAAAACGA	TGTCGAAGCA	AAACTTACTC	TTACCTGTTG	GGTTTCCTTC	CTTCCGACCT	1140
	TCTCTCTACC	CTTGCCTCCG	ATCGGTCTTA	TAGAACCTGT	ACTACAAACAC	TCCCCGGAGGA	1200
30	AAACCTCAACC	GAGGACTTTC	CGTGGTGGAT	ACTTATATCC	TTCTCTCGCC	TTCTGGAAA	1260
	GACATCTCGG	AAGAAGAGTA	CTTGAAGGCC	GCTATCCTCG	GTTGGTGTAT	CGAGCTTGT	1320
	CGCGTTTCT	TCATTACACCT	TTCTTCTCG	TCTTCTACTC	TCTTCTCTCG	AACTATCTTC	1380
	CCTGC GTGTC	ATCCTACACG	AATCTTATA	CTTACATGTT	GGAACATATG	CCCTGTTCTT	1440
35	AATTCAACCTC	TTTTGTCTCG	GATGGTAGCT	CCAAGCTTAC	TTCTTGGTGG	CTGATGATAT	1500
	GATGGACGCC	TCAATCACCC	GACGAGGCCA	ACCCCTGTTG	TACAAAGTTG	TTAGTCCCTT	1560
	CTTCTCTTTC	TGCCCTCTTT	CTTCTGAGCT	ATGCCAATTC	TTGATTGAAA	TCGGTGGTGC	1620
40	CGTCCGGACT	AATCCGTTTG	TCGTTTTAT	CATATCTCT	TGCACAAACA	GGAGGGAGTG	1680
	TCTAACATTG	CCATCAACGA	CGCGTTCATG	CTCGAGGGAG	CTATCTACTT	TTTGCTCAAG	1740
	AAGCAGTCTC	GAAAGCAGAG	CTACTATGTC	GATCTGCTAG	AGCTCTTCCA	CGATGTTGT	1800
45	CTCTATTTCT	TTTCTCCTC	CCCTCAATAA	ACTGTATTTG	TGACCATTCT	GGATCCTTTC	1860
	CTGACGATGA	ATCATCTTC	GGATGAGTAG	GTTACTTCC	AAACCGAGTT	GGGACAGCTC	1920
	ATCGATCTGT	TGACCGCTCC	TGAGGATCAC	GTGCGATCTCG	ACAAGTTCTC	CCTTAACAAAG	1980
50	TATGCCCGTC	ATATATTCTGT	TTTGTGCA	TCACGTCTGA	TTGTCAGCTC	CGATTATTGA	2040
	CTCTGATGGT	GATGGTATTG	ACCACATCAT	GCGATGTTG	ACTTCTCGT	AGGCACCACC	2100
	TCATCGTTGT	TTACAAGACC	GCTTCTATT	CATTCTACCT	TCCTGTCGCA	CTCGCTATGC	2160

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	GAATGGTGGG TCTCTCTCTT CAACTGTTCT TCCTGATTTC CTTGACCATC TGTAACATAA	2220
5	ATCCTTGGAA TTTTGAACTC TATGTCATAG GTCGGCGTGA CAGATGAGGA GGCCTACAAG	2280
	CTTGCCTCT CGATCCTCAT CCCGATGGGT GAATACTTTC AAGTTCAAGGA TGATGTGCTC	2340
	GACCGCGTTCG CTCCCTCCGGA GATCCTTGGGA AAGATCGGAA CCGACATCTT GGTGCGTTTT	2400
10	CGTTCCCTCC TTCTACGTTTC TGTTTCTAT CTTCTGACTC CCCGTCCATC ATTTATGCTT	2460
	CTGTTAAAAC GTATTGAAAC ATCAAAAGGA CAACAAATGT TCATGGCCTA TCAACCTTGC	2520
	ACTCTCTCTC GCCTCGCCCG CTCAGCGAGA GATTCTCGAT ACTTCGTACG GTCAGAAGAA	2580
	CTCGGAGGCA GAGGCCAGAG TCAAGGCTCT GTACGCTGAG CTTGATATCC AGGGAAAGTT	2640
15	CAACGCTTAT GAGTATGTCA TCTTTTTAA ATTTTCTAAT TTTCTTTCA TCTCTTGTTC	2700
	CCAAGAATTAA TTTTGTGAAA GTTCTGGGAC TGAACATGGT GCATCCCTTT GGTTCACTC	2760
	CGCATATGTC TCCCCTTGA ATAGGCAACA GAGTTACGAG TCGCTGAACA AGTTGATTGA	2820
20	CAGTATTGAC GAAGAGAAGA GTGGACTCAA GAAAGAAGTC TTCCACAGCT TCCTGGTAA	2880
	GGTCTATAAG CGAACGAAGT ATTCTCTC TTTATATGCA AAGGGAAAGAT TTTGGGGGA	2940
	GTGATAGGTA GGAAGAGAAG GGAGGGTCAT ATTCAATTAGG CATTCTCTT GCAGATATAG	3000
	ATGATCAAAA AGGGATATCG GTCTCTTCT TTGTTCCGAA TACATAATAA GTCATACGAA	3060
25	GCCGAACATG ACAAAAGTGG TTCATGAGAT CAAACTTTT GCATGATCTT CTGCATTTC	3120
	GTACAATTCT CTCCGCATCCT ATTAGGATCG ACCAGGAGA AGATGAGAGA AGGAAACCCT	3180
	CACCCCGTCA GATAACAAAC GAGAAGTCTC ATCACACACA CACACAGATG AAAGAGAAAA	3240
30	ATAAACTGAC GAGGATAACT TCCAATCCGA TTTTCCAGC CCACGAACCT TCCTGGTCC	3300
	CCGCTCCGGT GCCTCGAGT CCGATCAATG GGGCCAAAC GCCTGAAGAT CCAAAGAACCC	3360
	CTTGTTGAGG TGTATTTCTC GTCTGAGCAA TCTTAGATCC TTCAATTGCA AGTCGCGCAT	3420
35	ATATACCATC AACATCATCG TCATCACCCT CATTGTCGTC CACAACAGCA CGCAACGCC	3480
	GTTAATGGCA GGGCTTGGAC AACTTGAGGC GTTTCTAGC AGGTGGGACC GATTGGAGCT	3540
	CGACCCAGGG TGCACATCAC CAAGACACAT TCTCCTCAA ATGAGCGAAC AAGACATAAT	3600
40	GAGGGAAGTA GTACGCTATC GAACGTCTTC TCACATCCCG GGTTCTTGGC GTATCTTTG	3660
	GCGATTCTTT TTGTTGAAAT AGAAAATTGA AGAGAAAAAA AGAGATCCAC ATGATGAAGA	3720
	ACGGCTCTGT AGATTTCATGC TCGAAGAGAA GAAAGAAAGA AAAAGAGGGG AAGGAACCGA	3780
45	TCTGAATCTG TGGCCAACCA AAAAGTAGGC ACAAAAGATGA CAACAGCGCC CTCTTCGACA	3840
	AGTCTTGAA CTGCTTGTGG ATGAGACAAG TCCCAGCAGA TCAACATTCC TGCTTTACCC	3900
	CATGGAGTAT CAAACACCTG AGAATAGGTC TTGCCCGGCT GTAGATAATC TCTGGACCGT	3960
50	CATATGCGCG AAACGATCAG TACGACCGAC TCTACTCGAA GTCGTCAAGA GCACGGACGA	4020
	GAACGAAAAG AGGACAAACC GCTCTGGATG CCATAAATT CTCTTCAT ACCTCTCCCA	4080
	CCCACCCCTCA GG	4092

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 1091 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

10 (A) ORGANISM: Phaffia rhodozyma
 (B) STRAIN: ATCC96594

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

15	Met Tyr Thr Ile Lys His Ser Asn Phe Leu Ser Gln Thr Ile Ser Thr	1	5	10	15
20	Gln Ser Thr Thr Ser Trp Val Val Asp Ala Phe Phe Ser Leu Gly Ser	20	25	30	
25	Arg Tyr Leu Asp Leu Ala Lys Gln Ala Asp Ser Ala Asp Ile Phe Met	35	40	45	
30	Val Leu Leu Gly Tyr Val Leu Met His Gly Thr Phe Val Arg Leu Phe	50	55	60	
35	Leu Asn Phe Arg Arg Met Gly Ala Asn Phe Trp Leu Pro Gly Met Val	65	70	75	80
40	Leu Val Ser Ser Ser Phe Ala Phe Leu Thr Ala Leu Leu Ala Ala Ser	85	90	95	
45	Ile Leu Asn Val Pro Ile Asp Pro Ile Cys Leu Ser Glu Ala Leu Pro	100	105	110	
50	Phe Leu Val Leu Thr Val Gly Phe Asp Lys Asp Phe Thr Leu Ala Lys	115	120	125	
55	Ser Val Phe Ser Ser Pro Glu Ile Ala Pro Val Met Leu Arg Arg Lys	130	135	140	
60	Pro Val Ile Gln Pro Gly Asp Asp Asp Asp Leu Glu Gln Asp Glu His	145	150	155	160
65	Ser Arg Val Ala Ala Asn Lys Val Asp Ile Gln Trp Ala Pro Pro Val	165	170	175	
70	Ala Ala Ser Arg Ile Val Ile Gly Ser Val Glu Lys Ile Gly Ser Ser	180	185	190	
75	Ile Val Arg Asp Phe Ala Leu Glu Val Ala Val Leu Leu Gly Ala	195	200	205	
80	Ala Ser Gly Leu Gly Gly Leu Lys Glu Phe Cys Lys Leu Ala Ala Leu	210	215	220	
85	Ile Leu Val Ala Asp Cys Cys Phe Thr Phe Thr Phe Tyr Val Ala Ile	225	230	235	240
90	Leu Thr Val Met Val Glu Val His Arg Ile Lys Ile Ile Arg Gly Phe	245	250	255	

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	Arg Pro Ala His Asn Asn Arg Thr Pro Asn Thr Val Pro Ser Thr Pro			
	260	265	270	
5	Thr Ile Asp Gly Gln Ser Thr Asn Arg Ser Gly Ile Ser Ser Gly Pro			
	275	280	285	
	Pro Ala Arg Pro Thr Val Pro Val Trp Lys Lys Val Trp Arg Lys Leu			
	290	295	300	
10	Met Gly Pro Glu Ile Asp Trp Ala Ser Glu Ala Glu Ala Arg Asn Pro			
	305	310	315	320
	Val Pro Lys Leu Lys Leu Leu Ile Leu Ala Phe Leu Ile Leu His			
	325	330	335	
15	Ile Leu Asn Leu Cys Thr Pro Leu Thr Glu Thr Ala Ile Lys Arg			
	340	345	350	
	Ser Ser Ser Ile His Gln Pro Ile Tyr Ala Asp Pro Ala His Pro Ile			
	355	360	365	
20	Ala Gln Thr Asn Thr Thr Leu His Arg Ala His Ser Leu Val Ile Phe			
	370	375	380	
	Asp Gln Phe Leu Ser Asp Trp Thr Thr Ile Val Gly Asp Pro Ile Met			
	385	390	395	400
	Ser Lys Trp Ile Ile Ile Thr Leu Gly Val Ser Ile Leu Leu Asn Gly			
	405	410	415	
25	Phe Leu Leu Lys Gly Ile Ala Ser Gly Ser Ala Leu Gly Pro Gly Arg			
	420	425	430	
	Ala Gly Gly Gly Gly Ala Ala Ala Ala Ala Val Leu Leu Gly Ala			
	435	440	445	
30	Trp Glu Ile Val Asp Trp Asn Asn Glu Thr Glu Thr Ser Thr Asn Thr			
	450	455	460	
	Pro Ala Gly Pro Pro Gly His Lys Asn Gln Asn Val Asn Leu Arg Leu			
	465	470	475	480
35	Ser Leu Glu Arg Asp Thr Gly Leu Leu Arg Tyr Gln Arg Glu Gln Ala			
	485	490	495	
	Tyr Gln Ala Gln Ser Gln Ile Leu Ala Pro Ile Ser Pro Val Ser Val			
	500	505	510	
40	Ala Pro Val Val Ser Asn Gly Asn Ala Ser Lys Ser Ile Glu			
	515	520	525	
	Lys Pro Met Pro Arg Leu Val Val Pro Asn Gly Pro Arg Ser Leu Pro			
	530	535	540	
45	Glu Ser Pro Pro Ser Thr Thr Glu Ser Thr Pro Val Asn Lys Val Ile			
	545	550	555	560
	Ile Gly Gly Pro Ser Asp Arg Pro Ala Leu Asp Gly Leu Ala Asn Gly			
	565	570	575	
50	Asn Gly Ala Val Pro Leu Asp Lys Gln Thr Val Leu Gly Met Arg Ser			
	580	585	590	
	Ile Glu Glu Cys Glu Glu Ile Met Lys Ser Gly Leu Gly Pro Tyr Ser			
	595	600	605	

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Leu Asn Asp Glu Glu Leu Ile Leu Leu Thr Gln Lys Gly Lys Ile Pro
610 615 620

5 Pro Tyr Ser Leu Glu Lys Ala Leu Gln Asn Cys Glu Arg Ala Val Lys
625 630 635 640

Ile Arg Arg Ala Val Ile Ser Arg Ala Ser Val Thr Lys Thr Leu Glu
645 650 655

10 Thr Ser Asp Leu Pro Met Lys Asp Tyr Asp Tyr Ser Lys Val Met Gly
660 665 670

Ala Cys Cys Glu Asn Val Val Gly Tyr Met Pro Leu Pro Val Gly Ile
675 680 685

15 Ala Gly Pro Leu Asn Ile Asp Gly Glu Val Val Pro Ile Pro Met Ala
690 695 700

Thr Thr Glu Gly Thr Leu Val Ala Ser Thr Ser Arg Gly Cys Lys Ala
705 710 715 720

Leu Asn Ala Gly Gly Val Thr Thr Val Ile Thr Gln Asp Ala Met
725 730 735

20 Thr Arg Gly Pro Val Val Asp Phe Pro Ser Val Ser Gln Ala Ala Gln
740 745 750

Ala Lys Arg Trp Leu Asp Ser Val Glu Gly Met Glu Val Met Ala Ala
755 760 765

25 Ser Phe Asn Ser Thr Ser Arg Phe Ala Arg Leu Gln Ser Ile Lys Cys
770 775 780

Gly Met Ala Gly Arg Ser Leu Tyr Ile Arg Leu Ala Thr Ser Thr Gly
785 790 795 800

30 Asp Ala Met Gly Met Asn Met Ala Gly Lys Gly Thr Glu Lys Ala Leu
805 810 815

Glu Thr Leu Ser Glu Tyr Phe Pro Ser Met Gln Ile Leu Ala Leu Ser
820 825 830

35 Gly Asn Tyr Cys Ile Asp Lys Lys Pro Ser Ala Ile Asn Trp Ile Glu
835 840 845

Gly Arg Gly Lys Ser Val Val Ala Glu Ser Val Ile Pro Gly Ala Ile
850 855 860

40 Val Lys Ser Val Leu Lys Thr Thr Val Ala Asp Leu Val Asn Leu Asn
865 870 875 880

Ile Lys Lys Asn Leu Ile Gly Ser Ala Met Ala Gly Ser Ile Gly Gly
885 890 895

45 Phe Asn Ala His Ala Ser Asp Ile Leu Thr Ser Ile Phe Leu Ala Thr
900 905 910

Gly Gln Asp Pro Ala Gln Asn Val Glu Ser Ser Met Cys Met Thr Leu
915 920 925

Met Glu Ala Val Asn Asp Gly Lys Asp Leu Leu Ile Thr Cys Ser Met
930 935 940

50 Pro Ala Ile Glu Cys Gly Thr Val Gly Gly Thr Phe Leu Pro Pro
945 950 955 960

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Gln Asn Ala Cys Leu Gln Met Leu Gly Val Ala Gly Ala His Pro Asp
965 970 975

5 Ser Pro Gly His Asn Ala Arg Arg Leu Ala Arg Ile Ile Ala Ala Ser
980 985 990

Val Met Ala Gly Glu Leu Ser Leu Met Ser Ala Leu Ala Ala Gly His
995 1000 1005

10 Leu Ile Lys Ala His Met Lys His Asn Arg Ser Thr Pro Ser Thr Pro
1010 1015 1020

Leu Pro Val Ser Pro Leu Ala Thr Arg Pro Asn Thr Pro Ser His Arg
1025 1030 1035 1040

15 Ser Ile Gly Leu Leu Thr Pro Met Thr Ser Ser Ala Ser Val Ala Ser
1045 1050 1055

Met Phe Ser Gly Phe Gly Ser Pro Ser Thr Ser Ser Leu Lys Thr Val
1060 1065 1070

Gly Ser Met Ala Cys Val Arg Glu Arg Gly Asp Glu Thr Ser Val Asn
1075 1080 1085

20 Val Asp Ala
1090

(2) INFORMATION FOR SEQ ID NO:7:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 467 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

35 (vi) ORIGINAL SOURCE:
(A) ORGANISM: Phaffia rhodozyma
(B) STRAIN: ATCC96594

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Tyr Thr Ser Thr Thr Glu Gln Arg Pro Lys Asp Val Gly Ile Leu
1 5 10 15

40 Gly Met Glu Ile Tyr Phe Pro Arg Arg Ala Ile Ala His Lys Asp Leu
20 25 30

Glu Ala Phe Asp Gly Val Pro Ser Gly Lys Tyr Thr Ile Gly Leu Gly
35 40 45

45 Asn Asn Phe Met Ala Phe Thr Asp Asp Thr Glu Asp Ile Asn Ser Phe
50 55 60

Ala Leu Asn Ala Val Ser Gly Leu Leu Ser Lys Tyr Asn Val Asp Pro
65 70 75 80

50 Lys Ser Ile Gly Arg Ile Asp Val Gly Thr Glu Ser Ile Ile Asp Lys
85 90 95

Ser Lys Ser Val Lys Thr Val Leu Met Asp Leu Phe Glu Ser His Gly
100 105 110

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	Asn Thr Asp Ile Glu Gly Ile Asp Ser Lys Asn Ala Cys Tyr Gly Ser			
	115	120	125	
5	Thr Ala Ala Leu Phe Asn Ala Val Asn Trp Ile Glu Ser Ser Ser Trp			
	130	135	140	
	Asp Gly Arg Asn Ala Ile Val Phe Cys Gly Asp Ile Ala Ile Tyr Ala			
	145	150	155	160
10	Glu Gly Ala Ala Arg Pro Ala Gly Gly Ala Gly Ala Cys Ala Ile Leu			
	165	170	175	
	Ile Gly Pro Asp Ala Pro Val Val Phe Glu Pro Val His Gly Asn Phe			
	180	185	190	
15	Met Thr Asn Ala Trp Asp Phe Tyr Lys Pro Asn Leu Ser Ser Glu Tyr			
	195	200	205	
	Pro Ile Val Asp Gly Pro Leu Ser Val Thr Ser Tyr Val Asn Ala Ile			
	210	215	220	
20	Asp Lys Ala Tyr Glu Ala Tyr Arg Thr Lys Tyr Ala Lys Arg Phe Gly			
	225	230	235	240
	Gly Pro Lys Thr Asn Gly Val Thr Asn Gly His Thr Glu Val Ala Gly			
	245	250	255	
	Val Ser Ala Ala Ser Phe Asp Tyr Leu Leu Phe His Ser Pro Tyr Gly			
	260	265	270	
25	Lys Gln Val Val Lys Gly His Gly Arg Leu Leu Tyr Asn Asp Phe Arg			
	275	280	285	
	Asn Asn Pro Asn Asp Pro Val Phe Ala Glu Val Pro Ala Glu Leu Ala			
	290	295	300	
30	Thr Leu Asp Met Lys Lys Ser Leu Ser Asp Lys Asn Val Glu Lys Ser			
	305	310	315	320
	Leu Ile Ala Ala Ser Lys Ser Ser Phe Asn Lys Gln Val Glu Pro Gly			
	325	330	335	
35	Met Thr Thr Val Arg Gln Leu Gly Asn Leu Tyr Thr Ala Ser Leu Phe			
	340	345	350	
	Gly Ala Leu Ala Ser Leu Phe Ser Asn Val Pro Gly Asp Glu Leu Val			
	355	360	365	
40	Gly Lys Arg Ile Ala Leu Tyr Ala Tyr Gly Ser Gly Ala Ala Ala Ser			
	370	375	380	
	Phe Tyr Ala Leu Lys Val Lys Ser Ser Thr Ala Phe Ile Ser Glu Lys			
	385	390	395	400
45	Leu Asp Leu Asn Asn Arg Leu Ser Asn Met Lys Ile Val Pro Cys Asp			
	405	410	415	
	Asp Phe Val Lys Ala Leu Lys Val Arg Glu Glu Thr His Asn Ala Val			
	420	425	430	
50	Ser Tyr Ser Pro Ile Gly Ser Leu Asp Asp Leu Trp Pro Gly Ser Tyr			
	435	440	445	
	Tyr Leu Gly Glu Ile Asp Ser Met Trp Arg Arg Gln Tyr Lys Gln Val			
	450	455	460	

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Pro Ser Ala
465

5 (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 432 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Phaffia rhodozyma
(B) STRAIN: ATCC96594

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Glu Glu Ile Leu Val Ser Ala Pro Gly Lys Val Ile Leu Phe Gly
1 5 10 15

20 Glu His Ala Val Gly His Gly Val Thr Gly Ile Ala Ala Ser Val Asp
20 25 30

Leu Arg Cys Tyr Ala Leu Leu Ser Pro Thr Ala Thr Thr Thr Ser
35 40 45

25 Ser Ser Leu Ser Ser Thr Asn Ile Thr Ile Ser Leu Thr Asp Leu Asn
50 55 60

Phe Thr Gln Ser Trp Pro Val Asp Ser Leu Pro Trp Ser Leu Ala Pro
65 70 75 80

30 Asp Trp Thr Glu Ala Ser Ile Pro Glu Ser Leu Cys Pro Thr Leu Leu
85 90 95

Ala Glu Ile Glu Arg Ile Ala Gly Gln Gly Gly Asn Gly Gly Glu Arg
100 105 110

35 Glu Lys Val Ala Thr Met Ala Phe Leu Tyr Leu Leu Val Leu Leu Ser
115 120 125

Lys Gly Lys Pro Ser Glu Pro Phe Glu Leu Thr Ala Arg Ser Ala Leu
130 135 140

40 Pro Met Gly Ala Gly Leu Gly Ser Ser Ala Ala Leu Ser Thr Ser Leu
145 150 155 160

Ala Leu Val Phe Leu Leu His Phe Ser His Leu Ser Pro Thr Thr Thr
165 170 175

Gly Arg Glu Ser Thr Ile Pro Thr Ala Asp Thr Glu Val Ile Asp Lys
180 185 190

45 Trp Ala Phe Leu Ala Glu Lys Val Ile His Gly Asn Pro Ser Gly Ile
195 200 205

Asp Asn Ala Val Ser Thr Arg Gly Gly Ala Val Ala Phe Lys Arg Lys
210 215 220

50 Ile Glu Gly Lys Gln Glu Gly Gly Met Glu Ala Ile Lys Ser Phe Thr
225 230 235 240

Ser Ile Arg Phe Leu Ile Thr Asp Ser Arg Ile Gly Arg Asp Thr Arg
 245 250 255
 5 Ser Leu Val Ala Gly Val Asn Ala Arg Leu Ile Gln Glu Pro Glu Val
 260 265 270
 Ile Val Pro Leu Leu Glu Ala Ile Gln Ile Ala Asp Glu Ala Ile
 275 280 285
 10 Arg Cys Leu Lys Asp Ser Glu Met Glu Arg Ala Val Met Ile Asp Arg
 290 295 300
 Leu Gln Asn Leu Val Ser Glu Asn His Ala His Leu Ala Ala Leu Gly
 305 310 315 320
 15 Val Ser His Pro Ser Leu Glu Glu Ile Ile Arg Ile Gly Ala Asp Lys
 325 330 335
 Pro Phe Glu Leu Arg Thr Lys Leu Thr Gly Ala Gly Gly Gly Cys
 340 345 350
 Ala Val Thr Leu Val Pro Asp Asp Phe Ser Thr Glu Thr Leu Gln Ala
 355 360 365
 20 Leu Met Glu Thr Leu Val Gln Ser Ser Phe Ala Pro Tyr Ile Ala Arg
 370 375 380
 Val Gly Gly Ser Gly Val Gly Phe Leu Ser Ser Thr Lys Ala Asp Pro
 385 390 395 400
 25 Glu Asp Gly Glu Asn Arg Leu Lys Asp Gly Leu Val Gly Thr Glu Ile
 405 410 415
 Asp Glu Leu Asp Arg Trp Ala Leu Lys Thr Gly Arg Trp Ser Phe Ala
 420 425 430
 30

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 401 amino acids
 (B) TYPE: amino acid
 35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

40 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Phaffia rhodozyma
 (B) STRAIN: ATCC96594

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Val His Ile Ala Thr Ala Ser Ala Pro Val Asn Ile Ala Cys Ile
 1 5 10 15

45 Lys Tyr Trp Gly Lys Arg Asp Thr Lys Leu Ile Leu Pro Thr Asn Ser
 20 25 30

Ser Leu Ser Val Thr Leu Asp Gln Asp His Leu Arg Ser Thr Thr Ser
 35 40 45

50 Ser Ala Cys Asp Ala Ser Phe Glu Lys Asp Arg Leu Trp Leu Asn Gly
 50 55 60

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	Ile Glu Glu Glu Val Lys Ala Gly Gly Arg Leu Asp Val Cys Ile Lys			
65	70	75	80	
5	Glu Met Lys Lys Leu Arg Ala Gln Glu Glu Lys Asp Ala Gly Leu			
	85	90	95	
	Glu Lys Leu Ser Ser Phe Asn Val His Leu Ala Ser Tyr Asn Asn Phe			
	100	105	110	
10	Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Gly Leu Ala Ala Leu			
	115	120	125	
	Val Ala Ser Leu Ala Ser Leu Tyr Asn Leu Pro Thr Asn Ala Ser Glu			
	130	135	140	
15	Leu Ser Leu Ile Ala Arg Gln Gly Ser Gly Ser Ala Cys Arg Ser Leu			
	145	150	155	160
	Phe Gly Gly Phe Val Ala Trp Glu Gln Gly Lys Leu Ser Ser Gly Thr			
	165	170	175	
20	Asp Ser Phe Ala Val Gln Val Glu Pro Arg Glu His Trp Pro Ser Leu			
	180	185	190	
	His Ala Leu Ile Cys Val Val Ser Asp Glu Lys Lys Thr Thr Ala Ser			
	195	200	205	
	Thr Ala Gly Met Gln Thr Thr Val Asn Thr Ser Pro Leu Leu Gln His			
	210	215	220	
25	Arg Ile Glu His Val Val Pro Ala Arg Met Glu Ala Ile Thr Gln Ala			
	225	230	235	240
	Ile Arg Ala Lys Asp Phe Asp Ser Phe Ala Lys Ile Thr Met Lys Asp			
	245	250	255	
30	Ser Asn Gln Phe His Ala Val Cys Leu Asp Ser Glu Pro Pro Ile Phe			
	260	265	270	
	Tyr Leu Asn Asp Val Ser Arg Ser Ile Ile His Leu Val Thr Glu Leu			
	275	280	285	
35	Asn Arg Val Ser Val Gln Ala Gly Gly Pro Val Leu Ala Ala Tyr Thr			
	290	295	300	
	Phe Asp Ala Gly Pro Asn Ala Val Ile Tyr Ala Glu Glu Ser Ser Met			
	305	310	315	320
40	Pro Glu Ile Ile Arg Leu Ile Glu Arg Tyr Phe Pro Leu Gly Thr Ala			
	325	330	335	
	Phe Glu Asn Pro Phe Gly Val Asn Thr Glu Gly Gly Asp Ala Leu Arg			
	340	345	350	
45	Glu Gly Phe Asn Gln Asn Val Ala Pro Val Phe Arg Lys Gly Ser Val			
	355	360	365	
	Ala Arg Leu Ile His Thr Arg Ile Gly Asp Gly Pro Arg Thr Tyr Gly			
	370	375	380	
50	Glu Glu Glu Ser Leu Ile Gly Glu Asp Gly Leu Pro Lys Val Val Lys			
	385	390	395	400
	Ala			

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(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 355 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Phaffia rhodozyma
(B) STRAIN: ATCC96594

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Thr Thr Pro Glu Glu Lys Lys Ala Ala Arg Ala Lys Phe Glu
1 5 10 15

Ala Val Phe Pro Val Ile Ala Asp Glu Ile Leu Asp Tyr Met Lys Gly
20 25 30

Glu Gly Met Pro Ala Glu Ala Leu Glu Trp Met Asn Lys Asn Leu Tyr
35 40 45

Tyr Asn Thr Pro Gly Gly Lys Leu Asn Arg Gly Leu Ser Val Val Asp
50 55 60

Thr Tyr Ile Leu Leu Ser Pro Ser Gly Lys Asp Ile Ser Glu Glu Glu
65 70 75 80

Tyr Leu Lys Ala Ala Ile Leu Gly Trp Cys Ile Glu Leu Leu Gln Ala
85 90 95

Tyr Phe Leu Val Ala Asp Asp Met Met Asp Ala Ser Ile Thr Arg Arg
100 105 110

Gly Gln Pro Cys Trp Tyr Lys Val Glu Gly Val Ser Asn Ile Ala Ile
115 120 125

Asn Asn Ala Phe Met Leu Glu Gly Ala Ile Tyr Phe Leu Leu Lys Lys
130 135 140

His Phe Arg Lys Gln Ser Tyr Tyr Val Asp Leu Leu Glu Leu Phe His
145 150 155 160

Asp Val Thr Phe Gln Thr Glu Leu Gly Gln Leu Ile Asp Leu Leu Thr
165 170 175

Ala Pro Glu Asp His Val Asp Leu Asp Lys Phe Ser Leu Asn Lys His
180 185 190

His Leu Ile Val Val Tyr Lys Thr Ala Phe Tyr Ser Phe Tyr Leu Pro
195 200 205

Val Ala Leu Ala Met Arg Met Val Gly Val Thr Asp Glu Glu Ala Tyr
210 215 220

Lys Leu Ala Leu Ser Ile Leu Ile Pro Met Gly Glu Tyr Phe Gln Val
225 230 235 240

Gln Asp Asp Val Leu Asp Ala Phe Arg Pro Pro Glu Ile Leu Gly Lys
245 250 255

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Ile Gly Thr Asp Ile Leu Asp Asn Lys Cys Ser Trp Pro Ile Asn Leu
260 265 270

5 Ala Leu Ser Pro Ala Ser Pro Ala Gln Arg Glu Ile Leu Asp Thr Ser
275 280 285

Tyr Gly Gln Lys Asn Ser Glu Ala Glu Ala Arg Val Lys Ala Leu Tyr
290 295 300

10 Ala Glu Leu Asp Ile Gln Gly Lys Phe Asn Ala Tyr Glu Gln Gln Ser
305 310 315 320

Tyr Glu Ser Leu Asn Lys Leu Ile Asp Ser Ile Asp Glu Glu Lys Ser
325 330 335

Gly Leu Lys Lys Glu Val Phe His Ser Phe Leu Gly Lys Val Tyr Lys
340 345 350

15 Arg Ser Lys
355

20 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGNAARTAYA CNATHGGNYT NGGNCA

26

35 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TANARNWNS WNGTRTACAT RTTNCC

26

50 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

55

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

5 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

10 GAAGAACCCC ATCAAAAGCC TCGA

24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

25 AAAAGCCTCG AGATCCTTGT GAGCG

25

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

35 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGAAGCCAGA AGAGAAAA

18

40

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

45 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

55

TCGTCGAGGA AAGTAGAT

18

5 (2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGTACCATAT GTATCCTTCT ACTACCGAAC

30

20 (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCATGCGGAT CCTCAAGCAG AAGGGACCTG

30

35 (2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 32 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCNTGYTGYG ARAAYGTNAT HGGNTAYATG CC

32

50 (2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 32 base pairs
 (B) TYPE: nucleic acid

55

(C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

10 ATCCARTTDA TNGCNGCNGG YTTYTTRTCN GT

32

(2) INFORMATION FOR SEQ ID NO:21:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

25 GGCCATTCCA CACTTGATGC TCTGC

25

(2) INFORMATION FOR SEQ ID NO:22:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

40 GGCGGATATC TTTATGGTCC T

21

(2) INFORMATION FOR SEQ ID NO:23:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

50 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGTACCGAAG AAATTATGAA GAGTGG

26

5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

10

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTGCAGTCAG GCATCCACGT TCACAC

26

20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

25

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GCNCCNGGNA ARGTNATHYT NTTYGGNGA

29

35

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

40

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCCCANGTNS WNACNGCRTT RTCNACNCC

29

45

50

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs

55

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ACATGCTGTA GTCCATG

17

(2) INFORMATION FOR SEQ ID NO:28:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ACTCGGATTC CATGGA

16

(2) INFORMATION FOR SEQ ID NO:29:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Phaffia rhodozyma
 (B) STRAIN: ATCC96594

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TTGTTGTCGT AGCAGTGGGT GAGAG

25

45 (2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

55

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

5 GGAAGAGGAA GAGAAAAG

18

(2) INFORMATION FOR SEQ ID NO:31:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

20 TTGCCGAAC T CAATGTAG

18

(2) INFORMATION FOR SEQ ID NO:32:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Phaffia rhodozyma
- (B) STRAIN: ATCC96594

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

40 GGATCCATGA GAGCCCCAAA AGAAGA

26

(2) INFORMATION FOR SEQ ID NO:33:

45 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Phaffia rhodozyma
- (B) STRAIN: ATCC96594

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

55

GTCGACTCAA GCAAAAGACC AACGAC

26

(2) INFORMATION FOR SEQ ID NO:34:

5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

HTNAARTAYT TGGGNAARMG NGA

23

(2) INFORMATION FOR SEQ ID NO:35:

20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCRTTNGGNC CNGCRTCRAA NGTRTANGC

29

(2) INFORMATION FOR SEQ ID NO:36:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CCGAACCTCTC GCTCATCGCC

20

(2) INFORMATION FOR SEQ ID NO:37:

50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single

55

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

5 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

10 CAGATCAGCG CGTGGAGTGA

20

(2) INFORMATION FOR SEQ ID NO:38:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

25 CARGCNTAYT TYYTNGTNGC NGAYGA

26

(2) INFORMATION FOR SEQ ID NO:39:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

35 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

40 CAYTTRTTCT CYTGDARTC NGTNCCDATY TT

32

(2) INFORMATION FOR SEQ ID NO:40:

45 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

50 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

55

ATCCTCATCC CGATGGGTGA ATACT

25

5 (2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

15 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

20 AGGAGCGGTC AACAGATCGA TGAGC

25

25 (2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

35 GAATTCAAT GTCCACTACG CCTGA

25

40 (2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

55 GTCGACGGTA CCTATCACTC CCGCC

25

Claims

1. An isolated DNA sequence, which codes for an enzyme involved in the mevalonate pathway or the pathway from isopentenyl pyrophosphate to farnesyl pyrophosphate.
- 5 2. An isolated DNA sequence according to claim 1, wherein said enzyme has an activity selected from the group consisting of 3-hydroxy-3-methylglutaryl-CoA synthase activity, 3-hydroxy-3-methylglutaryl-CoA reductase activity, mevalonate kinase activity, mevalonate pyrophosphate decarboxylase activity and farnesyl pyrophosphate synthase activity.
- 10 3. An isolated DNA sequence according to claim 1 or 2, which is characterized in that
 - (a) the said DNA sequence codes for the said enzyme having an amino acid sequence selected from the group consisting of those described in SEQ ID NOs: 6, 7, 8, 9 and 10, or
 - (b) the said DNA sequence codes for a variant of the said enzyme selected from (i) an allelic variant, and (ii) an enzyme having one or more amino acid addition, insertion, deletion and/or substitution and having the stated enzyme activity.
- 15 4. An isolated DNA sequence according to any one of claims 1-3, which can be derived from a gene of *Phaffia rhodozyma* and is selected from:
 - (i) a DNA sequence represented in SEQ ID NOs: 1, 2, 4 or 5;
 - (ii) an isocoding or an allelic variant for the DNA sequence represented in SEQ ID NOs: 1, 2, 4 or 5; and
 - 20 (iii) a derivative of a DNA sequence represented in SEQ ID NOs: 1, 2, 4 or 5 with addition, insertion, deletion and/or substitution of one or more nucleotide(s), and coding for a polypeptide having the said enzyme activity.
- 25 5. An isolated DNA sequence, which is selected from:
 - (i) a DNA sequence represented in SEQ ID NO: 3;
 - (ii) an isocoding or an allelic variant for the DNA sequence represented in SEQ ID NO: 3; and
 - 30 (iii) a derivative of a DNA sequence represented in SEQ ID NO: 3 with addition, insertion, deletion and/or substitution of one or more nucleotide(s), and coding for a polypeptide having the mevalonate kinase activity.
- 35 6. An isolated DNA sequence as claimed in claim 1 or 2 and which is selected from:
 - (i) a DNA sequence which hybridizes under standard conditions with a sequence as shown in SEQ ID Nos: 1 - 10 or its complementary strand or fragments thereof; and
 - (ii) a DNA sequence which do not hybridize as defined in (i) because of the degeneration of the genetic code but which codes for polypeptides having exactly the same amino acid sequence as shown in SEQ ID Nos: 1 - 10 or those encoded by a DNA sequence as defined above under (i).
- 40 7. A vector or plasmid comprising a DNA sequence as defined in any of claims 1-6.
8. A host cell which has been transformed or transfected by a DNA sequence as claimed in anyone of claims 1 to 6, or a vector or plasmid as claimed in claim 7.
- 45 9. A process for producing an enzyme involved in the mevalonate pathway or the pathway from isopentenyl pyrophosphate to farnesyl pyrophosphate, which comprises culturing a host cell as claimed in claim 8, under the conditions conducive to the production of said enzyme.
- 50 10. A process for the production of isoprenoids or carotenoids, preferably astaxanthin, which comprises cultivating a host cell as claimed in claim 8 under suitable culture conditions.

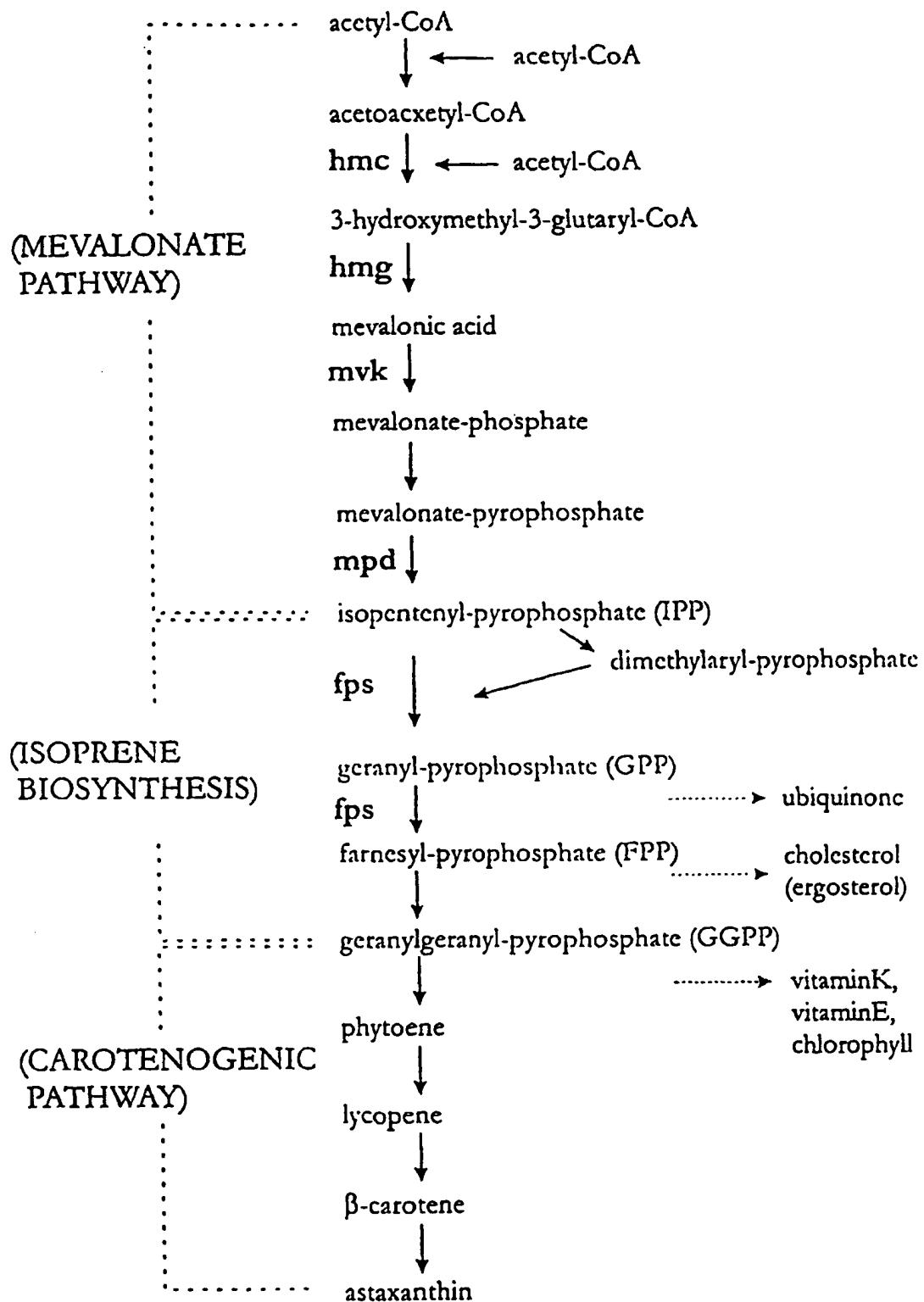
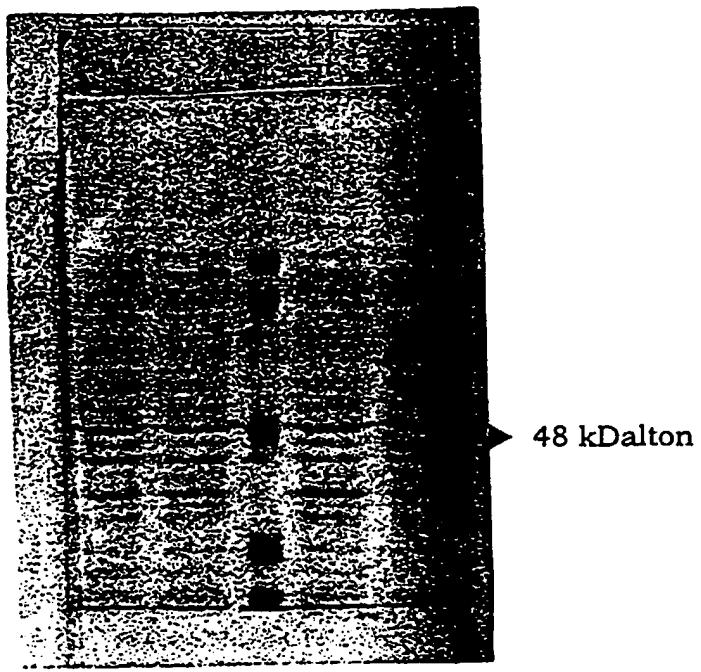


Fig. 1 Biosynthetic pathway from acetyl-CoA to astaxanthin in *P. rhodozyma*



Lane : 1 2 3 4 5

Fig. 2 Expression study of pseudo-mvk gene
by insertion of one base